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Contract Report

JUNE 1992

An Investigation Conducted by
Dr. Eve Riser-Roberts

IN SITU/ON-SITE BIODEGRADATION OF REFINED OILS AND FUELS (A Technology Review)

VOLUME 2

APPENDIX A

SUPPLEMENTARY TEXT

NAVAL CIVIL ENGINEERING LABORATORY PORT HUENEME CALIFORNIA 93043-5003

METRIC CONVERSION FACTORS

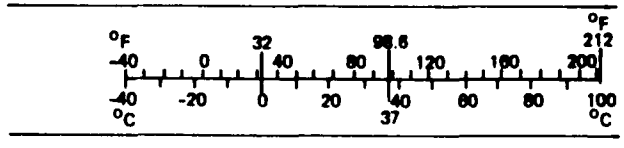
Approximate Conversions to Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
		LENGTH		
in	inches	*2.5	centimeters	cm
ft	feet	30	centimeters	cm
yd	yards	0.9	meters	m
mi	miles	1.6	kilometers	km
		AREA		
in^2	square inches	6.5	square centimeters	cm^2
ft^2	square feet	0.09	square meters	m^2
yd^2	square yards	0.8	square meters	m^2
mi^2	square miles	2.6	square kilometers	km^2
	acres	0.4	hectares	ha
		MASS (weight)		
oz	ounces	28	grams	g
lb	pounds	0.45	kilograms	kg
	short tons (2,000 lb)	0.9	tonnes	t
		VOLUME		
tsp	teaspoons	5	milliliters	ml
Tbsp	tablespoons	15	milliliters	ml
fl oz	fluid ounces	30	milliliters	ml
c	cups	0.24	liters	l
pt	pints	0.47	liters	l
qt	quarts	0.95	liters	l
gal	gallons	3.8	liters	l
ft^3	cubic feet	0.03	cubic meters	m^3
yd^3	cubic yards	0.76	cubic meters	m^3
		TEMPERATURE (exact)		
°F	Fahrenheit temperature	5/9 (after subtracting 32)	Celsius temperature	°C

*1 in = 2.54 (exactly). For other exact conversions and more detailed tables, see NBS Misc. Publ. 286, Units of Weights and Measures, Price $2.25, SD Catalog No. C13.10:286.

Approximate Conversions from Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
		LENGTH		
mm	millimeters	0.04	inches	in
cm	centimeters	0.4	inches	in
m	meters	3.3	feet	ft
m	meters	1.1	yards	yd
km	kilometers	0.6	miles	mi
		AREA		
cm^2	square centimeters	0.16	square inches	in^2
m^2	square meters	1.2	square yards	yd^2
km^2	square kilometers	0.4	square miles	mi^2
ha	hectares (10,000 m^2)	2.5	acres	
		MASS (weight)		
g	grams	0.035	ounces	oz
kg	kilograms	2.2	pounds	lb
t	tonnes (1,000 kg)	1.1	short tons	
		VOLUME		
ml	milliliters	0.03	fluid ounces	fl oz
l	liters	2.1	pints	pt
l	liters	1.06	quarts	qt
l	liters	0.26	gallons	gal
m^3	cubic meters	35	cubic feet	ft^3
m^3	cubic meters	1.3	cubic yards	yd^3
		TEMPERATURE (exact)		
°C	Celsius temperature	9/5 (then add 32)	Fahrenheit temperature	°F



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This extensive literature review covers all aspects of in situ and on-site bioremediation of gasoline, middle distillate fuels, and other refined petroleum products, using information available through 1988. Topics covered include: composition of different fuels, factors affecting biodegradation rates, responsible microorganisms, degradation pathways, factors enhancing biodegradation, and potential limitations. This report is in three volumes. Volume 1 covers general information of most interest to managers and non-specialists. Volume 2 contains technical information required for in-depth coverage. Volume 3 covers related information, including detailed applied information on screening and microbial enumberation methods, laboratory microorganisms studies, bioreclamation site preparation, bioreactor use and selection, cost data, and competing technologies.

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SECTION 1

INTRODUCTION

1.1 BACKGROUND

1.1.1 Environmental Contamination

1.1.2 Groundwater Contamination

1.1.3 Pollution Legislation

Table A.1-1 lists the classes of compounds established by the U.S. Environmental Protection Agency as organic priority pollutants (Leisinger, 1983)

The organic priority pollutants have been detected in the groundwater of all 10 EPA regions (Plumb, 1985). Table A.1-2 presents some of the most common contaminants.

Table A.1-1. Organic Priority Pollutants According to the U.S. Environmental Protection Agency (Leisinger, 1983)

Chemical Class	Number of Compounds
Aliphatics	3
Halogenated aliphatics	31
Nitrosamines	3
Aromatics	14
Chloroaromatics (including TCDD)	16
Polychlorinated biphenyls (PCBs)	7
Nitroaromatics	7
Polynuclear aromatic hydrocarbons	16
Pesticides and metabolites (including DDT)	17

Table A.1-2. Some of t Most Frequently Detected Groundwater Contaminants within Three Classes of Organic Priority Pollutants (Plumb, 1985)

	Detection Frequency (%)
Volatile Compounds	
Acetone	12.4
Acid Compounds	
Phenol	13.6
4-Methyl phenol	5.8
2-Methyl phenol	4.2
2,4-Dimethylphenol	1.9
Benzoic acid	1.3
Base/Neutral Compounds	
Naphthalene	4.1

Based upon a compilation of data from 183 disposal sites located across the U.S. All frequencies expressed in percent.

1.2 BIODEGRADATION AS A TREATMENT ALTERNATIVE

1.2.1 On-site Biological Treatment Techniques

The biological process reactors available for water and wastewater treatment can be classified according to the nature of their biological growth (Sutton, 1987). Those in which the active biomass is suspended as free organisms or microbial aggregates can be regarded as suspended growth reactors, whereas, those in which growth occurs on or within a solid medium can be termed supported growth or fixed-film reactors.

1.2.1.1 Aerobic Biological Systems

Aerobic treatment systems include conventional activated sludge processes, as well as modifications, such as sequencing batch reactors, and aerobic attached growth biological processes, such as rotating biological contactors (RBCs) and trickling filters (Roberts, Koff, and Karr, 1988). Aerobic processes are capable of significantly reducing a wide range of organic toxic and hazardous compounds; however, treatment is limited to dilute aqueous wastes (usually not exceeding 1 percent). Genetically engineered bacteria have been recently developed for effective biological treatment of specific hazardous wastes that are relatively uniform in composition. Such systems are typically used to treat aqueous wastes contaminated with low levels of nonhalogenated organic or certain halogenated organics. This treatment requires consistent, stable operating conditions.

1.2.1.1.1 Activated Sludge

There are many variations of the conventional activated sludge process, all of which use the same principles of unit operation (Roberts, Koff, and Karr, 1988). The first step in the process involves aeration in open tanks, in which the organic biodegradable matter in the waste is degraded by microorganisms in the presence of oxygen. The hydraulic detention time of this process is usually from 6 to 24 hr, depending upon the process mode. This is followed by a sludge-liquid separation step in a clarifier. The organisms multiply during the process. A zoogleal sludge is settled out and a portion of the organisms (Return Activated Sludge, or RAS) is recycled to the aeration basin, which allows growth of an acclimated population. The remaining sludge is wasted, while the clarified water is discharged in a manner appropriate to its quality. Organic loading rates can vary from 10 to 180 lb of BOD applied per 1000 cf, depending upon the mixed liquor suspended solids (MLSS) concentration, the food-to-microorganism (F/M) ratio, and oxygen supply. Variations of the conventional activated sludge system that utilize pure oxygen or oxygen-enriched air, instead of air, produce a more rapid breakdown of chemical solutes. Extended aeration involves longer detention times than conventional activated sludge and relies on a higher population of microorganisms. Contact stabilization involves only short contact of the aqueous wastes and suspended microbial solids, with subsequent settling of sludge and treatment of the sludge to eliminate the sorbed organics. Use of powdered activated carbon is also reported to have excellent pollutant removal capabilities for wastes that are difficult to treat.

Activated sludge has a great potential for treatment of hazardous waste, since it can be easily controlled (Shuckrow, Pajak, and Touhill, 1982). Howev-

er, nonbiodegradable organics and metals can be adsorbed by the biomass, interfere with metabolism, and cause the generated sludge to be hazardous. Activated sludge processes can handle organic loadings as high as 10,000 ppm BOD, but are sensitive to shock loads (JRB Associates, Inc., 1982). See Appendix B for further discussion of the use of activated sludge.

1.2.1.1.2 Trickling Filters or Fixed-film Systems

Trickling filters or fixed-film systems involve contact of the aqueous waste stream with microorganisms attached to some inert medium, such as rock or specially designed plastic material (Roberts, Koff, and Karr, 1988). The original trickling filter consisted of a bed of rocks over which the contaminated water was sprayed. Microbial deposits form slime layers on the rocks where metabolism of the solute organics occurs. Oxygen is provided with the air being introduced countercurrently to the wastewater flow. Present technology suggests, however, that gas-suspended biomass systems are better applicable to treating oily sludges than are fixed- film systems. Trickling filters are not as effective as activated sludge, but are less sensitive to shock loads and have lower energy costs (Lee and Ward, 1986). Their principal use is for secondary treatment or as a roughing filter to even out loading (JRB Associates, Inc., 1982).

In fixed-film processes, the cell retention time is long compared with suspended growth processes (Stratton, 1981; Switzenbaum and Jewell, 1980). Fixed-film processes foster long cell retention and enhance growth of slow-growing microorganisms. Such populations are particularly advantageous when sorption is the main mechanism for the removal of a compound. One advantage of these processes is that they can provide cell concentrations of an order of magnitude higher than those found in suspended growth systems. A study of the partitioning of organic compounds into biomass indicates that efficient removal is possible only when the biomass concentration is large (Stratton, 1981; Matter-Muller, Gujer, Giger, and Strumm, 1980).

The subsurface environment is generally characterized by low substrate and nutrient concentrations and high specific surface area, which favor predominance of bacteria attached to solid surfaces in the form of biofilms (Bouwer and McCarty, 1984). Attached bacteria have an advantage over suspended bacteria, as they can remain near the source of fresh substrate and nutrients contained in groundwater that flows by them.

1.2.1.1.3 Biological Towers

Biological towers are a modification of the trickling filter (Roberts, Koff, and Karr, 1988). The medium (e.g., of polyvinyl chloride, polyethylene, polystyrene, or redwood) is stacked into towers, which typically reach 16 to 20 ft. The wastewater is sprayed across the top, and as it moves downward, air is drawn upward through the tower. A slime layer of microorganisms forms on the medium and removes the organic contaminants as the water flows over the slime layer.

1.2.1.1.4 Rotating Biological Contactor (RBC)

A rotating biological contactor consists of a series of rotating discs, connected by a shaft set in a basin or trough, as in Figure A.1-1 (Roberts,

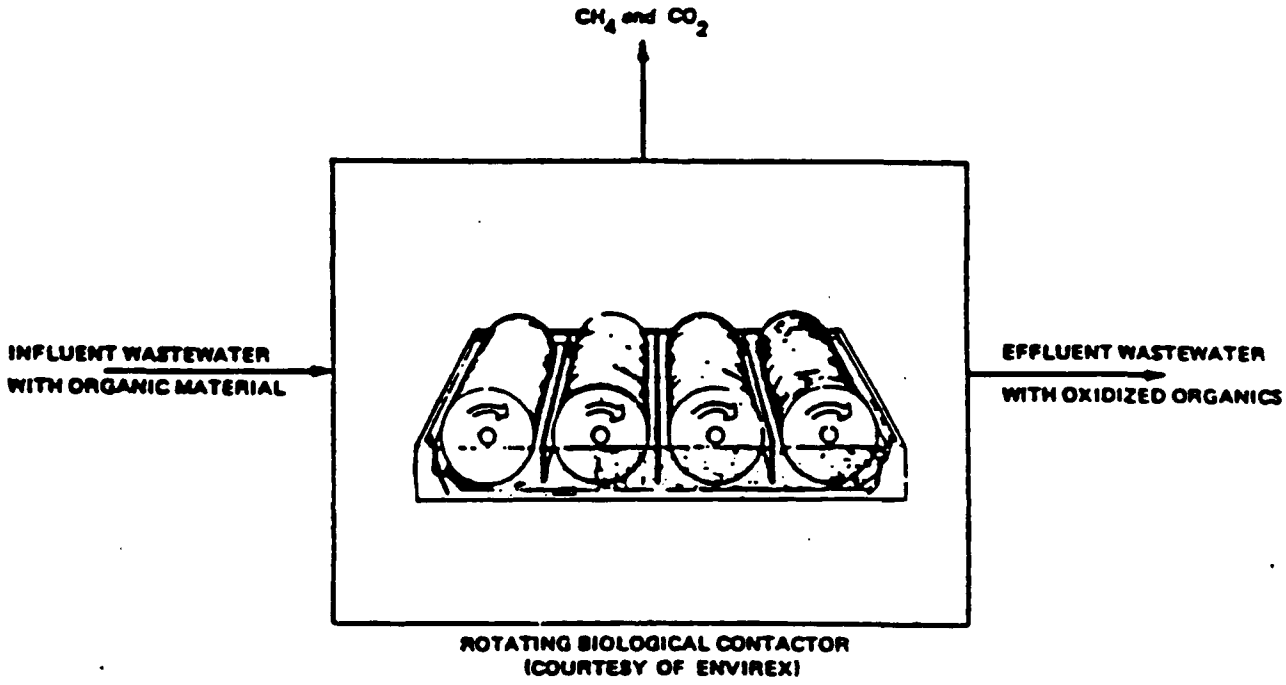


Figure A.1-1. Diagram of Rotating Biological Contactor (Roberts, Koff, and Karr, 1988)

Koff, and Karr, 1988). The contaminated water passes through the basin where the microorganisms, attached to the discs, metabolize the organics present in the water. Approximately, 40 percent of the disc's surface area is submerged. This allows the slime layer to alternately come into contact with the contaminated water and the air where the oxygen is provided to the microorganisms. These units are compact, and they can handle large flow variations and high organic shock loads. They do not require the use of aeration equipment. Due to the varied composition of oily sludges and high concentrations of solids, oils, and heavy metals, the applicability of the RBC to this problem material is questionable.

1.2.1.1.5 Lagoons and Waste Stabilization Ponds

Lagoons and waste stabilization ponds are similar to activated sludge processes, except for the biomass recycle (Wilkinson, Kelso, and Hopkins, 1978). Lagoons provide dilution and buffering of load fluctuations; but they require more land, and the operational controls are less flexible (Shuckrow, Pajak, and Touhill, 1982). Oxygen can be supplied by aerating the lagoons to speed up the degradation (Johnson, 1978). Anaerobic or facultative lagoons are also available, with their ease of operation and low costs, but these will produce a lower quality effluent (JRB Associates, Inc., 1982; Johnson, 1978). They utilize anaerobic degradation pathways, which may be more efficient for the removal of some compounds, and sludge production is minimized.

Waste stabilization ponds are principally a polishing technique useful for low organic wastewaters (Johnson, 1978). Since natural biodegradation processes are employed, requirements for energy and chemical additions are low; however, large land areas are needed. Appendix B discusses the use of sewage lagoons for biodegradation.

1.2.1.1.6 Fluidized-bed Reactors

Particles, such as sand or coal, are fluidized by the action of the aeration gas stream and the wastewater stream and are colonized by a dense growth of microorganisms, which gives rapid treatment (McCarty, Rittmann, and Bouwer, 1984). This treatment process is largely experimental at present and is discussed in Appendix B.

1.2.1.2 Anaerobic Digesters

Anaerobic biological digester systems promote the reduction of organic matter to methane and carbon dioxide in an oxygen-free environment (Lee and Ward, 1986). The most common anaerobic attached growth treatment process is the anaerobic filter. This process consists of a column filled with solid media. A number of proprietary anaerobic biotechnology processes are on the market, each with distinct features, but all the processes involve the fundamental anaerobic bacterial conversion to methane. The digester gas can be flared or fired in boilers, gas turbines, or reciprocating engines with or without the prior removal of sulfurous gases. A typical anaerobic filter system is depicted in Figure A.1-2 (Lee and Ward, 1986).

These systems are used to treat aqueous wastes with low to moderate levels of organics. Anaerobic digestion can dechlorinate certain halogenated organics better than aerobic treatment. Stable, consistent operating conditions must be

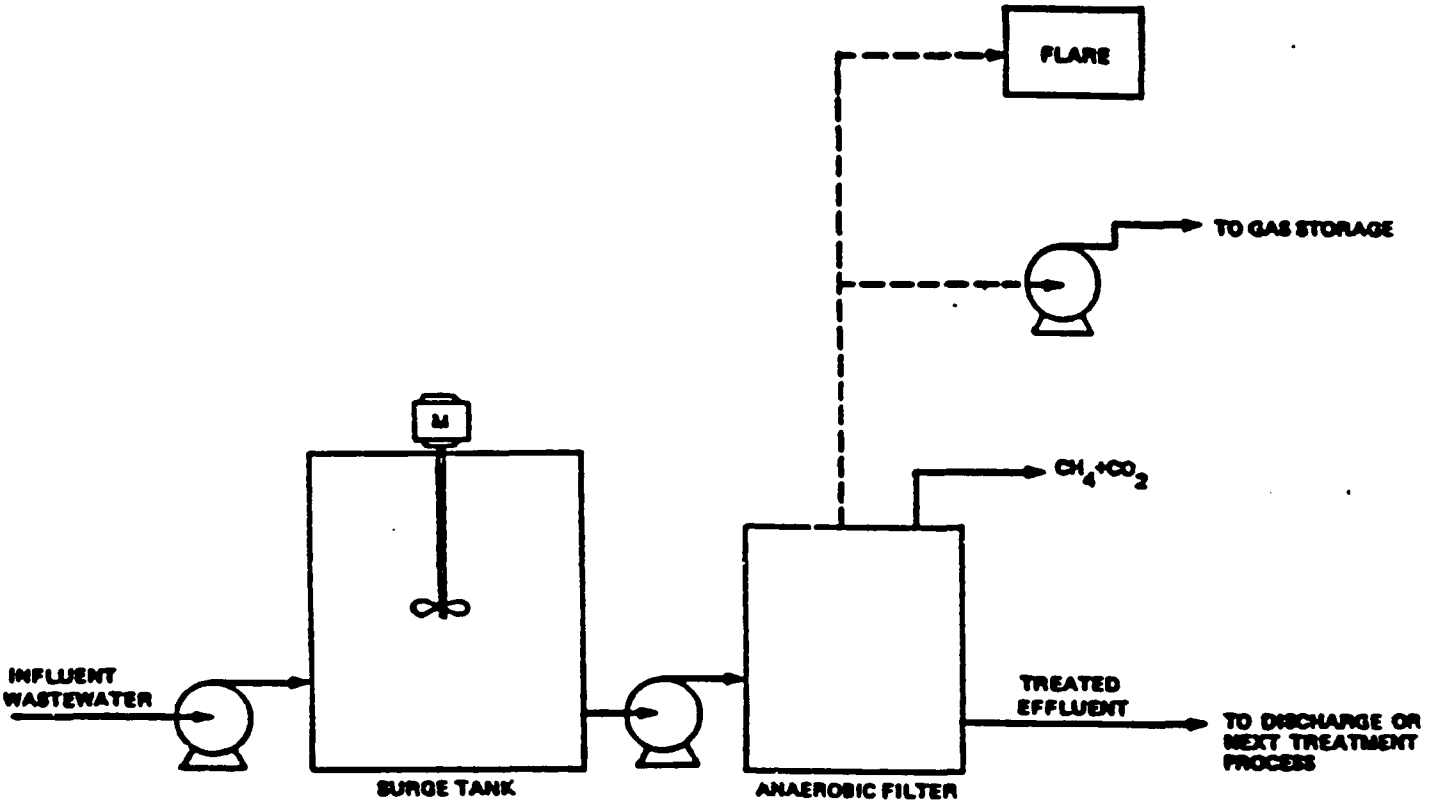


Figure A.1-2. Diagram of Typical Anaerobic Filter System (Lee and Ward, 1986)

maintained. Anaerobic degradation can take place in native soils; however, when used as a controlled treatment process, an air-tight reactor is required. Hazardous organic substances that have been found to be amenable to anaerobic treatment include acetaldehyde, acetic anhydride, acetone, acrylic acid, aniline, benzoic acid, butanol, creosol, ethyl acrylate, MEK, phenol, and vinyl acetate.

1.2.1.3 Landfarming

This process is described in Appendix B, Innovative Remedial Technologies.

1.2.2 In Situ Bioreclamation

SECTION 2

ORGANIC COMPOUNDS IN REFINED FUELS AND FUEL OILS

2.1 CHEMICAL COMPOSITION OF FUEL OILS

2.1.1 Naptha

2.1.2 Kerosene

2.1.3 Fuel Oil and Diesel #2

Table A.2-1 lists organic compounds found in diesel fuel #2, as reported by Clewell, 1981.

Table A.2-1. Composition of Diesel Fuel #2 (Clewell, 1981)

Component	Concentration (% Volume)	Component	Concentration (% Volume)
C_{10} paraffins	0.9	C_{15} paraffins	7.4
C_{10} cycloparaffins	0.6	C_{15} cycloparaffins	5.5
C_{10} aromatics	0.4	C_{15} aromatics	3.2
C_{11} paraffins	2.3	C_{16} paraffins	5.8
C_{11} cycloparaffins	1.7	C_{16} cycloparaffins	4.4
C_{11} aromatics	1.0	C_{16} aromatics	2.5
C_{12} paraffins	3.8	C_{17} paraffins	5.5
C_{12} cycloparaffins	2.8	C_{17} cycloparaffins	4.1
C_{12} aromatics	1.6	C_{17} aromatics	2.4
C_{13} paraffins	6.4	C_{18} paraffins	4.3
C_{13} cycloparaffins	4.8	C_{18} cycloparaffins	3.2
C_{13} aromatics	2.8	C_{18} aromatics	1.8
C_{14} paraffins	8.8	C_{19} paraffins	0.7
C_{14} cycloparaffins	6.6	C_{19} cycloparaffins	0.6
C_{14} aromatics	3.8	C_{19} aromatics	0.3

2.1.4 Gasoline

Tables A.2-2, A.2-3, and A.2-4 list the organic compounds and trace elements found in gasoline, as reported by different references.

Table A.2-2. Composition of Various Gasolines (Ghassemi, Panahloo, and Quinlivan, 1984b)

Paraffins		Olefins	
Propane		Dimethyl pentanes	
Isobutane ($1C_4$)		Methyl hexanes	
n-butane (nC_4)		Trimethyl pentanes	
Isopentane ($1C_5$)		Normal heptane	
n-pentane ($1C_5$)		Dimethyl hexanes	
Dimethyl butanes (C_6)		Methylethyl pentanes	
Methyl pentanes (C_6)		Dimethyl hexanes	
n-hexane		Trimethyl hexanes	
n-octane			
Naphthenes		**Olefins**	
Methylcyclopentane		Methylbutene	
Cyclohexane		Pentene	
Methylcyclohexane		Methylpentene	
Other cyclic saturates		Other olefins	
Aromatics			
Benzene		Propylbenzene	
Toluene		Methylethylbenzenes	
Ethylbenzene		Trimethylbenzene	
Xylenes		Other aromatics	

Table A.2-3. Components of Gasoline (Jamison, Raymond, and Hudson, 1976)

Component	Component
n-Propane	2,5-Dimethylhexane
n-Butane	2,4-Dimethylhexane
n-Pentane	2,3-Dimethylhexane
n-Hexane	3,4-Dimethylhexane
n-Heptane	2,2-Dimethylhexane
n-Octane	2,2-Dimethylheptane
n-cis-Butene-2	1,1-Dimethylcyclopentane
n-Pentane-2	1,2- and 1,3-Dimethylcyclopentane
2,3-Dimethylbutene-1	1,3- and 1,4-Dimethylcyclohexane
Olefins C_4	1,2-Dimethylcyclohexane
Olefins C_5	2,2,3-Trimethylbutane
Olefins C_6	2,2,4-Trimethylpentane
Isobutane	2,2,3-Trimethylpentane
Cyclopentane	2,3,4-Trimethylpentane
Cyclohexane	2,3,3-Trimethylpentane
Methylcyclopentane	2,2,5-Trimethylpentane
Methylcyclohexane	1,2,4-Trimethylcyclopentane
2-Methylbutane	Ethylpentane
2-Methylpentane	Ethylcyclopentane
3-Methylpentane	Ethylcyclohexane
2-Methylhexane	Benzene
3-Methylhexane	Ethylbenzene
2-Methylheptane	Toluene
3-Methylheptane	o-Xylene
4-Methylheptane	m-Xylene
2,2-Dimethylbutane	p-Xylene
2,3-Dimethylbutane	
2,2-Dimethylpentane	
2,4-Dimethylpentane	
3,3-Dimethylpentane	
2,3-Dimethylpentane	

Table A.2-4. Composition of Gasoline (State of California, 1987)

Compound	Number of Carbons	Concentration (Weight Percent)
Straight Chain Alkanes		
Propane	3	0.01 to 0.14
n-Butane	4	3.93 to 4.70
n-Pentane	5	5.75 to 10.92
n-Hexane	6	0.24 to 3.50
n-Heptane	7	0.31 to 1.96
n-Octane	8	0.36 to 1.43
n-Nonane	9	0.07 to 0.83
n-Decane	10	0.04 to 0.50
n-Undecane	11	0.05 to 0.22
n-Dodecane	12	0.04 to 0.09
Branched Alkanes		
Isobutane	4	0.12 to 0.37
2,2-Dimethylbutane	6	0.17 to 0.84
2,3-Dimethylbutane	6	0.59 to 1.55
2,2,3-Trimethylbutane	7	0.01 to 0.04
Neopentane	5	0.02 to 0.05
Isopentane	5	6.07 to 10.17
2-Methylpentane	6	2.91 to 3.85
3-Methylpentane	6	2.4 (vol)
2,4-Dimethylpentane	7	0.23 to 1.71
2,3-Dimethylpentane	7	0.32 to 4.17
3,3-Dimethylpentane	7	0.02 to 0.03
2,2,3-Trimethylpentane	8	0.09 to 0.23
2,2,4-Trimethylpentane	8	0.32 to 4.58
2,3,3-Trimethylpentane	8	0.05 to 2.28
2,3,4-Trimethylpentane	8	0.11 to 2.80
2,4-Dimethyl-3-ethyl-pentane	9	0.03 to 0.07
2-Methylhexane	7	0.36 to 1.48
3-Methylhexane	7	0.30 to 1.77
2,4-Dimethylhexane	8	0.34 to 0.82
2,5-Dimethylhexane	8	0.24 to 0.52
3,4-Dimethylhexane	8	0.16 to 0.37
3-Ethylhexane	8	0.01
2-Methyl-3-ethylhexane	9	0.04 to 0.13
2,2,4-Trimethylhexane	9	0.11 to 0.18
2,2,5-Trimethylhexane	9	0.17 to 5.89
2,3,3-Trimethylhexane	9	0.05 to 0.12
2,3,5-Trimethylhexane	9	0.05 to 1.09
2,4,4-Trimethylhexane	9	0.02 to 0.16

Table A.2-4. Composition of Gasoline (State of California, 1987) (Continued)

Compound	Number of Carbons	Concentration (Weight Percent)
2-Methylheptane	8	0.48 to 1.05
3-Methylheptane	8	0.63 to 1.54
4-Methylheptane	8	0.22 to 0.52
2,2-Dimethylheptane	9	0.01 to 0.08
2,3-Dimethylheptane	9	0.13 to 0.51
2,6-Dimethylheptane	9	0.07 to 0.23
3,3-Dimethylheptane	9	0.01 to 0.08
3,4-Dimethylheptane	9	0.07 to 0.33
2,2,4-Trimethylheptane	10	0.12 to 1.70
3,3,5-Trimethylheptane	10	0.02 to 0.06
3-Ethylheptane	10	0.02 to 0.16
2-Methyloctane	9	0.14 to 0.62
3-Methyloctane	9	0.34 to 0.85
4-Methyloctane	9	0.11 to 0.55
2,6-Dimethyloctane	10	0.06 to 0.12
2-Methylnonane	10	0.06 to 0.41
3-Methylnonane	10	0.06 to 0.32
4-Methylnonane	10	0.04 to 0.26
Cycloalkanes		
Cyclopentane	5	0.19 to 0.58
Methylcyclopentane	6	Not quantified
1-Methyl-cis-2-ethyl-cyclopentane	8	0.06 to 0.11
1-Methyl-trans-3-ethyl-cyclopentane	8	0.06 to 0.12
1-cis-2-dimethylcyclo-pentane	7	0.07 to 0.13
1-trans-2-dimethylcyclo-pentane	7	0.06 to 0.20
1,1,2-trimethylcyclo-pentane	8	0.06 to 0.11
1-trans-2-cis-3-tri-methylcyclopentane	8	0.01 to 0.25
1-trans-2-cis-4-tri-methylcyclopentane	8	0.03 to 0.16
Ethylcyclopentane	7	0.14 to 0.21
n-Propylcyclopentane	8	0.01 to 0.06
Isopropylcyclopentane	8	0.01 to 0.02
1-trans-3-dimethyl-cyclohexane	8	0.05 to 0.12
Ethylcyclohexane	8	0.17 to 0.42

Table A.2-4. Composition of Gasoline (State of California, 1987) (Continued)

Compound	Number of Carbons	Concentration (Weight Percent)
Straight Chain Alkenes		
cis-2-Butene	4	0.13 to 0.17
trans-2-Butene	4	0.16 to 0.20
Pentene-1	5	0.33 to 0.45
cis-2-Pentene	5	0.43 to 0.67
trans-2-Pentene	5	0.52 to 0.90
cis-2-Hexene	6	0.15 to 0.24
trans-2-Hexene	6	0.18 to 0.36
cis-3-Hexene	6	0.11 to 0.13
trans-3-Hexene	6	0.12 to 0.15
cis-3-Heptene	7	0.14 to 0.17
trans-2-Heptene	7	0.06 to 0.10
Branched Alkenes		
2-Methyl-1-butene	5	0.22 to 0.66
3-Methyl-1-butene	5	0.08 to 0.12
2-Methyl-2-butene	5	0.96 to 1.28
2,3-Dimethyl-1-butene	6	0.08 to 0.10
2-Methyl-1-pentene	6	0.20 to 0.22
2,3-Dimethyl-1-pentene	7	0.01 to 0.02
2,4-Dimethyl-1-pentene	7	0.02 to 0.03
4,4-Dimethyl-1-pentene	7	0.6 (vol)
2-Methyl-2-pentene	6	0.27 to 0.32
3-Methyl-cis-2-pentene	6	0.35 to 0.45
3-Methyl-trans-2-pentene	6	0.32 to 0.44
4-Methyl-cis-2-pentene	6	0.04 to 0.05
4-Methyl-trans-2-pentene	6	0.08 to 0.30
4,4-Dimethyl-cis-2-pentene	7	0.02
4,4-Dimethyl-trans-2-pentene	7	Not quantified
3-Ethyl-2-pentene	7	0.03 to 0.04
Cycloalkenes		
Cyclopentene	5	0.12 to 0.18
3-Methylcyclopentene	6	0.03 to 0.08
Cyclohexene	6	0.03
Alkyl Benzenes		
Benzene	6	0.12 to 3.50
Toluene	7	2.73 to 21.80

Table A.2-4. Composition of Gasoline (State of California, 1987) (Continued)

Compound	Number of Carbons	Concentration (Weight Percent)
o-Xylene	8	0.68 to 2.86
m-Xylene	8	1.77 to 3.87
p-Xylene	8	0.77 to 1.58
1-Methyl-4-ethylbenzene	9	0.18 to 1.00
1-Methyl-2-ethylbenzene	9	0.19 to 0.56
1-Methyl-3-ethylbenzene	9	0.31 to 2.86
1-Methyl-2-n-propyl-benzene	10	0.01 to 0.17
1-Methyl-3-n-propyl-benzene	10	0.08 to 0.56
1-Methyl-3-isopropyl-benzene	10	0.01 to 0.12
1-Methyl-3-t-butyl-benzene	11	0.03 to 0.11
1-Methyl-4-t-butyl-benzene	11	0.04 to 0.13
1,2-Dimethyl-3-ethyl-benzene	10	0.02 to 0.19
1,2-Dimethyl-4-ethyl-benzene	10	0.50 to 0.73
1,3-Dimethyl-2-ethyl-benzene	10	0.21 to 0.59
1,3-Dimethyl-4-ethyl-benzene	10	0.03 to 0.44
1,3-Dimethyl-5-ethyl-benzene	10	0.11 to 0.42
1,3-Dimethyl-5-t-butyl-benzene	12	0.02 to 0.16
1,4-Dimethyl-2-ethyl-benzene	10	0.05 to 0.36
1,2,3-Trimethylbenzene	9	0.21 to 0.48
1,2,4-Trimethylbenzene	9	0.66 to 3.30
1,3,5-Trimethylbenzene	9	0.13 to 1.15
1,2,3,4-Tetramethyl-benzene	10	0.02 to 0.19
1,2,3,5-Tetramethyl-benzene	10	0.14 to 1.06
1,2,4,5-Tetramethyl-benzene	10	0.05 to 0.67
Ethylbenzene	8	0.36 to 2.86
1,2-Diethylbenzene	10	0.57
1,3-Diethylbenzene	10	0.05 to 0.38
n-Propylbenzene	9	0.08 to 0.72
Isopropylbenzene	9	<0.01 to 0.23
n-Butylbenzene	10	0.04 to 0.44
Isobutylbenzene	10	0.01 to 0.08
sec-Butylbenzene	10	0.01 to 0.13
t-Butylbenzene	10	0.12
n-Pentylbenzene	11	0.01 to 0.14
Isopentylbenzene	11	0.07 to 0.17
Indan	9	0.25 to 0.34
1-Methylindan	10	0.04 to 0.17
2-Methylindan	10	0.02 to 0.10
4-Methylindan	10	0.01 to 0.16
5-Methylindan	10	0.09 to 0.30

Table A.2-4. Composition of Gasoline (State of California, 1987) (Continued)

Compound	Number of Carbons	Concentration (Weight Percent)
Tetralin	10	0.01 to 0.14
Polynuclear Aromatic Hydrocarbons		
Naphthalene	10	0.09 to 0.49
Pyrene	16	Not quantified
Benz(a)anthracene	18	Not quantified
Benz(a)pyrene	20	0.19 to 2.8 mg/kg
Benzo(e)pyrene	20	Not quantified
Benzo(g,h,i)perylene	21	Not quantified
Elements		
Bromine		80 to 345 ug/g
Cadmium		0.01 to 0.07 ug/g
Chlorine		80 to 300 ug/g
Lead		530 to 1120 ug/g
Sodium		<0.6 to 1.4 ug/g
Sulfur		0.10 to 0.15(ASTM)
Vanadium		<0.02 to 0.001 ug/g
Additives		
Ethylene dibromide		0.7 to 177.2 ppm
Ethylene dichloride		150 to 300 ppm
Tetramethyl lead		
Tetraethyl lead		

2.1.5 JP-5

Tables A.2-5, A.2-6, and A.2-7 list the organic compounds and trace elements found in JP-5, as reported by different references.

Table A.2-5. Selected Compound Types Occurring in JP-5 (Varga, Lieberman, and Avella, 1985)

Aromatic	Partial Saturation	Saturated
Benzene		Cyclohexane
Indene	Indane (Indan)	Hydrindane (Hydroindane)
Naphthalene	Tetralin (Tetrahydronaphthalene)	Decalin (Decahydronaphthalene)
Acenaphthalene	Acenaphthene	Perhydroacenaphthalene
Phenanthrene	Tetrahydrophenanthrene	Perhydrophenanthrene

Table A.2-6. Major Components of JP-5 (Smith, Harper, and Jaber, 1981)

Fuel Component	Concentration (Weight Percent)
n-Octane	0.12
1,3,5-Trimethylcyclohexane	0.09
1,1,3-Trimethylcyclohexane	0.05
m-Xylene	0.13
3-Methyloctane	0.07
2,4,6-Trimethylheptane	0.09
o-Xylene	0.09
n-Nonane	0.38
1,2,4-Trimethylbenzene	0.37
n-Decane	1.79
n-Butylcyclohexane	0.90
1,3-Diethylbenzene	0.61
1,4-Diethylbenzene	0.77
4-Methyldecane	0.78
2-Methyldecane	0.61
1-Ethylpropylbenzene	1.16
n-Undecane	3.95
2,6-Dimethyldecane	0.72
1,2,3,4-Tetramethylbenzene	1.48
Naphthalene	0.57
2-Methylundecane	1.39
n-Dodecane	3.94
2,6-Dimethylundecane	2.00
1,2,4-Triethylbenzene	0.72
2-Methylnaphthalene	0.90
1-Methylnaphthalene	1.44
1-Tridecene	0.45
Phenylcyclohexane	0.82
n-Tridecane	3.45
1-t-Butyl-3,4,5-trimethylbenzene	0.24
n-Heptylcyclohexane	0.99
n-Heptylbenzene	0.27
Biphenyl	0.70
1-Ethylnaphthalene	0.32
2,6-Dimethylnaphthalene	1.12
n-Tetradecane	2.72
2,3-Dimethylnaphthalene	0.46
n-Octylbenzene	0.78
n-Pentadecane	1.67
n-Hexadecane	1.07
n-Heptadecane	0.12

Table A.2-7. Trace Elements in Shale-derived JP-5 (Ghassemi, Panahloo, and Quinlivan, 1984a)

Element	ppm	Element	ppm	Element	ppm
Al	0.048	Cu	<0.02	Si	<=10
Sb	<3	Fe	<0.01	Ag	<0.02
As	<0.5	Pb	<0.06	Na	0.14
Be	<0.01	Mg	<5.3	Sr	<=0.94
Cd	<0.02	Mn	<0.02	Tl	<6
Ca	<=0.6	Hg	<2	Sn	<=0.93
Cl	<2	Mo	<=0.03	Ti	<0.4
Cr	<=0.094	Ni	<3.9	V	<=0.0008
Co	<=0.04	Se	<0.5	Zn	<=0.02

2.1.6 JP-4

Tables A.2-8, A.2-9, and A.2-10 list the organic compounds and trace elements found in JP-4, as reported by different references.

Table A.2-8. Composition of JP-4 (Clewell, 1981)

Component	Concentration (% Volume)	Component	Concentration (% Volume)
C_5 hydrocarbons	3.9	Napthalene	0.2
C_6 paraffins	8.1	C_{11} paraffins	4.8
C_6 cycloparaffins	2.1	C_{11} cycloparaffins	2.5
Benzene	0.3	Dicycloparaffins	3.4
C_7 paraffins	9.4	C_{11} aromatics	1.1
C_7 cycloparaffins	7.1	C_{11} napthalenes	0.2
Toluene	0.7	C_{12} paraffins	2.8
C_8 paraffins	10.1	C_{12} cycloparaffins	1.2
C_8 cycloparaffins	7.4	C_{12} aromatics	0.5
C_8 aromatics	1.6	C_{12} napthalenes	0.2
C_9 paraffins	9.1	C_{13} paraffins	1.1
C_9 cycloparaffins	4.3	C_{13} cycloparaffins	0.4
C_9 aromatics	2.4	C_{13} aromatics	0.1
C_{10} paraffins	7.3	C_{14} hydrocarbons	0.2
C_{10} cycloparaffins	3.7	C_{15} hydrocarbons	0.1
C_{10} aromatics	1.8	Tricycloparaffins	1.8
		Residual hydrocarbons	0.1

Table A.2-9. Major Components of JP-4 (Smith, Harper, and Jaber, 1981)

Fuel Component	Concentration (Weight Percent)
n-Butane	0.12
Isobutane	0.66
n-Pentane	1.06
2,2-Dimethylbutane	0.10
2-Methylpentane	1.28
3-Methylpentane	0.89
n-Hexane	2.21
Methylcyclopentane	1.16
2,2-Dimethylpentane	0.25
Benzene	0.50
Cyclohexane	1.24
2-Methylhexane	2.35
3-Methylhexane	1.97
trans-1,3-Dimethylcyclopentane	0.36
cis-1,3-Dimethylcyclopentane	0.34
cis-1,2-Dimethylcyclopentane	0.54
n-Heptane	3.67
Methylcyclohexane	2.27
2,2,3,3-Tetramethylbutane	0.24
Ethylcyclopentane	0.26
2,5-Dimethylhexane	0.37
2,4-Dimethylhexane	0.58
1,2,4-Trimethylcyclopentane	0.25
3,3-Dimethylhexane	0.26
1,2,3-Trimethylcyclopentane	0.25
Toluene	1.33
2,2-Dimethylhexane	0.71
2-Methylheptane	2.70
4-Methylheptane	0.92
cis-1,3-Dimethylcyclohexane	0.42
3-Methylheptane	3.04
1-Methyl-3-ethylcyclohexane	0.17
1-Methyl-2-ethylcyclohexane	0.39
Dimethylcyclohexane	0.43
n-Octane	3.80
1,3,5-Trimethylcyclohexane	0.99
1,1,3-Trimethylcyclohexane	0.48
2,5-Dimethylheptane	0.52
Ethylbenzene	0.37
m-Xylene	0.96
p-Xylene	0.35

Table A.2-9. Major Components of JP-4 (Smith, Harper, and Jaber, 1981) (Continued)

Fuel Component	Concentration (Weight Percent)
3,4-Dimethylheptane	0.43
4-Ethylheptane	0.18
4-Methyloctane	0.86
2-Methyloctane	0.88
3-Methyloctane	0.79
o-Xylene	1.01
1-Methyl-4-ethylcyclohexane	0.48
n-Nonane	2.25
Isopropylbenzene	0.30
n-Propylbenzene	0.71
1-Methyl-3-ethylbenzene	0.49
1-Methyl-4-ethylbenzene	0.43
1,3,5-Trimethylbenzene	0.42
1-Methyl-2-ethylbenzene	0.23
1,2,4-Trimethylbenzene	1.01
n-Decane	2.16
n-Butylcyclohexane	0.70
1,3-Diethylbenzene	0.46
1-Methyl-4-propylbenzene	0.40
1,3-Dimethyl-5-ethylbenzene	0.61
1-Methyl-2-i-propylbenzene	0.29
1,4-Dimethyl-2-ethylbenzene	0.70
1,2-Dimethyl-4-ethylbenzene	0.77
n-Undecane	2.32
1,2,3,4-Tetramethylbenzene	0.75
Naphthalene	0.50
2-Methylundecane	0.64
n-Dodecane	2.00
2,6-Dimethylundecane	0.71
2-Methylnaphthalene	0.56
1-Methylnaphthalene	0.78
n-Tridecane	1.52
2,6-Dimethylnaphthalene	0.25
n-Tetradecane	0.73

Table A.2-10. Trace Elements in Petroleum-based JP-4 (Ghassemi, Panahloo, and Quinlivan, 1984a)

Element	ppm	Element	ppm	Element	ppm
Al	NA	Cu	<0.05	Si	NA
Sb	<0.5	Fe	<0.05	Ag	NA
As	0.5	Pb	0.09	Na	NA
Be	NA	Mg	NA	Sr	NA
Cd	<0.03	Mn	NA	Th	NA
Ca	NA	Hg	<1	Sn	NA
Cl	NA	Mo	NA	Ti	NA
Cr	<0.05	Ni	<0.05	V	<0.05
Co	NA	Se	<0.03	Zn	<0.05

2.2 FACTORS AFFECTING CONTAMINATION AND BIODEGRADATION

The fate of toxic pollutants in the environment is determined by a variety of chemical, physical, biological, and environmental processes that interact in a complex manner (Pfaender, Shimp, Palumbo, and Bartholomew, 1985). The factors that appear to affect the overall chemical movement and availability for biodegradation in soil are 1) adsorption, 2) physical properties of the soil, and 3) climatic conditions (Panchal and Zajic, 1978). The four principal means for chemical transport within soils are 1) downward flowing water, 2) upward moving water, 3) diffusion in soil water, and 4) diffusion in the air space of the soil.

A common technique for measuring the biodegradability of an organic compound is calculation of the BOD/COD ratio. This ratio is an indication of the amount of degradation that occurs (BOD), relative to the amount of material available to be degraded (COD). In Table A.2-11 are relative biodegradabilities by adapted sludge cultures of various substances in terms of a BOD/COD ratio, after five days of incubation (Environmental Protection Agency, 1985b). A higher ratio represents a higher relative biodegradability. As the ratio approaches zero, the compound becomes less degradable.

2.2.1 Physical, Chemical, Biological, and Environmental Factors

2.2.1.1 Solubility

Organic compounds differ widely in their solubility, from infinitely miscible polar compounds, such as methanol, to extremely low solubility nonpolar compounds, such as high molecular weight polynuclear aromatic hydrocarbons (Horvath, 1982). Many synthetic chemicals have low water solubilities (Stucki and Alexander, 1987). The availability of a compound to an organism will dictate its biodegradability (Environmental Protection Agency, 1985b). Compounds with greater aqueous solubilities are generally more available to degradative enzymes. An example is cis-1,2-dichloroethylene, which is preferentially degraded relative to trans-1,2-dichloroethylene. This is probably due to "cis" being more polar than "trans" and, therefore, more water soluble. However, the rate of solubilization may not be the sole factor determining the degradation of lipophilic compounds (Thomas and Alexander, 1983). Surfactants can increase the solubility and, thus, the degradability of compounds. Table A.2-12 lists a number of common organic contaminants and relates their biodegradability to their solubility in water (Brubaker and O'Neill, 1982).

The main solute components of seawater are known to be inorganic salts (mainly NaCl), which are 35 percent of the total concentration. Thus, the seawater solubility of organic chemicals will be lower than distilled water solubility due to salting-out effects (Hashimoto, Tokura, Kishi, and Strachan, 1984). See Table A.2-13 for the solubilities of chemicals in seawater.

The interaction between the groundwater and an organic contaminant depends upon the solubility of the substance and the organic carbon content already present in the aquifer (Mackay, Roberts, and Cherry, 1985). Hydrophobic compounds that do not dissolve well in water and are attracted to solids will have a more retarded flow velocity than the water itself. The density and viscosity of the material will determine if it will float on the water table, mix with

Table A.2-11. BOD_5/COD Ratios for Various Organic Compounds (Environmental Protection Agency, 1985b)

Compound	Ratio
Relatively Undegradable	
Heptane	0
Hexane	0
o-Xylene	<0.008
m-Xylene	<0.008
Ethylbenzene	<0.009
Moderately Degradable	
Gasolines (various)	0.02
Nonanol	>0.033
Undecanol	<0.04
Dodecanol	0.097
p-Xylene	<0.11
Toluene	<0.12
Jet Fuels (various)	0.15
Kerosene	0.15
Relatively Degradable	
Naphthalene (molten)	<0.20
Hexanol	0.20
Benzene	<0.39
p-Xylene	<0.11
Toluene	<0.12
Jet Fuels (various)	0.15
Kerosene	0.15
Benzaldehyde	0.62
Phenol	0.81
Benzoic acid	0.84

Table A.2-12. Solubility and Biodegradability of Some Common Organic Contaminants (Brubaker and O'Neill, 1982)

Contaminant	Solubility in Water[a]	Biodegradability
Acetone	Miscible	++
Aniline	35 g/l	++
Anthracene	1 mg/l	+
Benzene	320 mg/l	++
o-Cresol	31 g/l	++
Isopropanol	Miscible	++
Methanol	Miscible	++
Methylene Chloride	20 g/l	(?)
Methylethylketone	370 g/l	++
Naphthalene	29 ug/ml[b]	nr
Phenol	82 g/l	+
Pyrene	0.2 mg/l	+
Toluene	470 mg/l	++
Benzo(a)pyrene[c]	3.8 g/l	nr

++ = readily biodegradable
+ = slow biodegradability
? = materials of uncertain biodegradability
nr = not reported
a = The actual solubility may be influenced greatly by other chemicals in the water.
b = (Thomas, Yordy, Amador, and Alexander, 1986)
c = (Mackay and Shiu, 1977)

Table A.2-13. Solubilities of Chemicals in Pacific Seawater at 20°C (Hashimoto, Tokura, Kishi, and Strachan, 1984)

Chemical	Solubility
Anthracene	1.18×10^{-7}
Pyrene	3.22×10^{-7}
Phenanthrene	4.15×10^{-6}
Biphenyl	2.39×10^{-5}
Naphthalene	1.34×10^{-4}
p-Nitrotoluene	1.83×10^{-3}
p-Toluidine	4.89×10^{-2}
o-Nitrophenol	8.34×10^{-3}
m-Nitrophenol	6.49×10^{-2}
p-Nitrophenol	7.76×10^{-2}
Phenol	1.35

groundwater, or sink. Slightly soluble compounds, such as benzene, will develop a plume in the saturated zone. Plumes containing different compounds may overlap. More information is needed about the rates and products of biotransformation in such superimposed plumes and the effects of complex contaminant distributions on the activities of microorganisms.

Organic compounds are only rarely found in groundwater at concentrations approaching their solubility limits (Mackay, Roberts, and Cherry, 1985). The observed concentrations are usually more than a factor of 10 lower than the solubility, presumably because of the diffusional limitations of dissolution and the dilution of the dissolved organic contaminants by dispersion. Therefore, the volume of groundwater contaminated by an organic liquid phase could be much larger than that calculated by assuming dissolution to the solubility limit.

Microorganisms use various mechanisms to metabolize organic substrates present at concentrations that exceed their water solubility (Thomas, Yordy, Amador, and Alexander, 1986). The physical state of the insoluble phase of a compound, whether it is a liquid or a solid, may affect its degradation. Liquid hydrocarbons can be taken up and incorporated into the cell membrane (Johnson, 1964); whereas, the mechanism of utilization of solid substrates is not fully understood. Polycyclic aromatic hydrocarbons might be used only in the dissolved state. Growth of pure cultures of bacteria on naphthalene, phenanthrene, and anthracene are faster on the solid substrates having the highest water solubilities (Wodzinski and Johnson, 1968). Rates of dissolution of naphthalene have been found to be directly related to its surface area (Thomas, Yordy, Amador, and Alexander, 1986).

Many microorganisms may excrete emulsifiers that increase the surface area of the substrate (Thomas, Yordy, Amador, and Alexander, 1986), or they may modify their cell surface to increase its affinity for hydrophobic substrates and, thus, facilitate their absorption (Kappeli, Walther, Mueller, and Fiechter, 1984). It has been suggested that microbial degradation of the insoluble phase of crystalline hydrocarbons is difficult because of the large amount of energy needed to disperse the solid (Zilber, Rosenberg, and Gutnick, 1980). If an organism cannot use the insoluble form of a chemical, it may be expected that the organism will first metabolize that portion of a chemical that is in solution and that the subsequent rate of transformation of the compound will be limited by the rate of dissolution. It has been found that increasing the surface area of hexadecane increases the microbial destruction of the alkane (Fogel, Lancione, Sewall, and Boethling, 1985). Emulsification can be employed for this purpose (Liu, 1980), and some organisms produce their own emulsifiers (Thomas, Yordy, Amador, and Alexander, 1986). Some microbes may even be able to utilize an insoluble substrate. Such an example is a marine pseudomonad, which was found to grow on bound and free n-tetracosane (Zilber, Rosenberg, and Gutnick, 1980).

While growth of bacteria appears to be limited by the rate of dissolution of a hydrocarbon, exponential growth does not always continue in parallel with the available material (Stucki and Alexander, 1987). Growth of strains of Flavobacterium and Beijerinckia in media containing 84 uM phenanthrene began to decline at densities of about 4×10^6/ml. The dissolution rate should have allowed exponential growth to a fivefold higher cell density. It is not clear why exponential growth ended so soon.

Many organic solutes with a low water solubility preferentially leave dilute water solutions and concentrate primarily in lipids of animals and in organic material of soil and sediments (Karickhoff, Brown, and Scott, 1979). This action is proportional to the partitioning between octanol-1 and water. The octanol-water partition coefficient (P) is defined as

$$P = C_O/C_W ,$$

where C_O and C_W are the concentration of the solute in n-octanol and water. P values measured in the laboratory can be used to predict the environmental behavior of organic pollutants (Mallon and Harrison, 1984). The hydrophobic nature of a pollutant, as measured by the octanol/water partition coefficient or by the dielectric constant, is important in predicting its flow through clay soils (Green, Lee, and Jones, 1981).

The subsurface transport of immiscible organic liquids is governed by a different set of factors than those for dissolved contaminants (Mackay, Roberts, and Cherry, 1985). The migration of an immiscible organic liquid phase is governed largely by its density and viscosity. Commonly encountered groundwater contaminants, including halogenated aliphatics with one or two carbon atoms, tend to have moderately low solubilities. These may migrate as discrete nonaqueous phases, with some components dissolving into the surrounding groundwater. The concentration of many organic compounds in groundwater is often limited by their very low solubilities; however, they may be toxic at very low concentrations (Freeze and Cherry, 1979). The presence of large quantities of high- density, low-solubility contaminants can provide a "hidden" source for long-term contamination of the groundwater.

The rate at which dissolved organic contaminants migrate in groundwaters is determined by the processes of advection, dispersion, sorption, chemical and biological transformations, and perhaps by volatilization at the water table (Barker and Patrick, 1985). All processes except advection can bring about lower aqueous concentrations and, therefore, can contribute to natural attenuation. Attenuation of organic chemicals by dilution in groundwater is not as great as in surface water because the flow velocities of groundwaters are generally low (Steelman and Ecker, 1984). Therefore, these contaminants tend to maintain much of their integrity as they move through an aquifer.

2.2.1.2 Advection

In sand and gravel aquifers, the dominant factor in the migration of a dissolved contaminant is advection, the process by which solutes are transported by the bulk motion of flowing groundwater (Mackay, Roberts, and Cherry, 1985). Groundwater generally flows from regions of the subsurface where water level is high to regions where water level is low, a process called hydraulic gradient. In most cases, the flow velocities under natural gradient conditions can be between 10 and 100 m/yr or even lower. In the zone of influence of a high-capacity well or wellfield, however, the artificially increased gradient substantially increases the local velocity.

2.2.1.3 Dispersion and Diffusion

The rate of movement of organic chemicals through air, water, and organic matter is directly proportional to the concentration of the toxicant and its diffusion coefficient (Kaufman, 1983).

Dissolved contaminants spread as they move with the groundwater (Mackay, Roberts, and Cherry, 1985). This dispersion results from molecular diffusion and mechanical mixing, and causes a net flux of the solutes from a zone of high concentration to a zone of lower concentration. This movement causes the concentrations to diminish with increasing distance from the source and the plume to become more uniform. Dispersion in the direction of flow is often much greater than dispersion in the directions transverse to the flow.

Percolating water is the principal means of movement of relatively nonvolatile chemicals, and diffusion in soil water is important only for transport over very small distances (Kaufman, 1983).

2.2.1.4 Sorption

Sorption is perhaps the most important single factor affecting the behavior of organic chemicals in the soil environment (Kaufman, 1983). Adsorption to soil constituents will affect the rate of volatilization, diffusion, or leaching, as well as the availability of chemicals to microbial or chemical degradation. Some dissolved contaminants may interact with the aquifer solids encountered along the flow path through physical adsorption, partitioning, ion exchange, (Freeze and Cherry, 1979), oppositely charged surfaces, or formation of a bond (Roberts and Valocchi, 1982).

Compounds that sorb strongly onto solids are retarded in their movement through an aquifer (Roberts and Valocchi, 1982). Sorption takes place because the compound has either a low affinity for water (hydrophobic) or has a high affinity for the solid. The advancing front of sorbing contaminants moves at a linear velocity that is slower than that of the carrier groundwater. The main subsurface solids responsible for adsorption of organic chemicals are solid organic matter (strong associations with hydrophobic organic compounds), clay minerals, and amorphous minerals (e.g., iron hydroxides) (Pettyjohn and Hounslow, 1983).

These interactions distribute the contaminants between the aqueous phase and the aquifer solids, diminish concentrations in the aqueous phase, and retard movement of the contaminant relative to groundwater flow (Rubin, 1983). The higher the fraction of contaminant sorbed, the more retarded is its transport. Also, the more hydrophobic a compound is, the more it should be retarded. Sorption equilibrium may require weeks or months and, thus, may not always be reached in the field (Karickhoff, 1984). The interaction of these variable factors make it difficult to accurately predict groundwater transport of the contaminants (Mackay, Roberts, and Cherry, 1985).

A large amount of organic material is necessary to adsorb low molecular weight hydrocarbons, such as chloroform. Highly water-soluble organic substances, such as acetone and methanol, are only slightly retarded by sorption, but fortunately are easily degraded (Pettyjohn and Hounslow, 1983).

The pKa, or dissociation constant, of a compound indicates the degree of acidity or basicity that a compound will exhibit and, therefore, should be very important in determining both the extent of adsorption and the ease of desorption (Kaufman, 1983). Functional groups can affect the degree of sorption of a chemical (Brindley and Thompson, 1966). Chain molecules terminating in -OH, in -COOH, and in $-NH_2$ readily form complexes with montmorillonite, whereas, similar molecules terminating in -Cl and -Br do not. All compounds are adsorbed strongly at low pH; anionic substances are adsorbed negatively at slightly basic conditions; and nonionic compounds are moderately adsorbed (Frissel, 1961). Adsorption processes are exothermic and desorption processes are endothermic, and an increase in temperature should reduce adsorption and favor the desorption process (Kaufman, 1983).

2.2.1.5 Volatility

The surface sheen residue emanating from an emulsified oil slick after evaporation of the light boiling components gradually becomes enriched in high molecular constituents concomitant with a change in chemical composition. Such constituents exhibit efficient absorption over a broad spectral range extending into the IR region. A film of crude oil will, therefore, absorb a considerable amount of solar energy, leading to transformation and degradation of the film through photoinduced oxidation. Petroleum resins have been shown to be particularly unstable in air and sunlight, and they may aromatize and evolve into structures more like asphaltenes (Tissot and Welte, 1978).

2.2.1.6 Viscosity

Viscosity and surface-wetting properties affect the transport of an organic liquid phase (Mackay, Roberts, and Cherry, 1985). Viscosity of polluting oils is an important property that determines, in part, the spreading and dispersion of the hydrocarbon mixture and, thus, the surface area available for microbial attack (Atlas, 1978c). It affects a chemical's migration in groundwater (Noel, Benson, and Beam, 1983). For example, about four times the volume of a light fuel oil in the high viscosity range would be retained by the average soil, compared with gasoline, a distillate with a lower viscosity. Gasoline would also spread over a wider area of an aquifer than a light fuel oil. Contaminants that are highly water soluble must be handled differently than those that float on the water table, like gasoline (Nielsen, 1983).

Large quantities of immiscible liquid organic contaminants could be stored as droplets dispersed within the pores of aquifer media, even if the bulk of the migrating mass of liquid is removed (Mackay, Roberts, and Cherry, 1985). These droplets may then dissolve over time into the groundwater flowing past them.

2.2.1.7 Density

Density differences of only 1 percent can influence fluid movement in the subsurface (Mackay, Roberts, and Cherry, 1985). The specific gravities of hydrocarbons (gasoline and other petroleum distillates) may be as low as 0.7, and halogenated hydrocarbons, are almost without exception, significantly more dense than water.

Density determines where in the aquifer the contaminant will most likely be concentrated. Low-density hydrocarbons have a tendency to float on water and may be found in the upper portions of an aquifer. High-density hydrocarbons would sink to the lower portions of the aquifer. It is important to recognize that the migration of dense organic liquids is largely uncoupled from the hydraulic gradient that drives advective transport and that the movement may have a dominant vertical component, even in horizontally flowing aquifers.

An organic liquid contaminant, such as gasoline, which is immiscible with and less dense than groundwater, would migrate vertically to and then float on the water table, spreading out in the downgradient direction (Mackay, Roberts, and Cherry, 1985). If the organic liquid contains a contaminant slightly soluble in water, e.g., benzene, a plume would form in the saturated zone. A complex pattern of overlapping plumes can develop when many contaminants are involved.

2.2.1.8 Chemical Structure

The structure, concentration, and toxicity of a chemical are important in determining whether it is accumulated in the environment and the environmental impact of the accumulation (Leisinger, 1983). The chemical will accumulate if its structure prevents mineralization or biodegradation by organisms. This may be due to its insolubility or to a novel chemical structure to which microorganisms have not been exposed during evolutionary history. Such compounds are termed xenobiotic. This name refers to compounds of anthropogenic (manmade) origin, as well as to compounds that may occur naturally but exceed normal levels in the environment. Various laboratory culture techniques applied to samples from nature can be used to select or develop bacteria with the ability to biodegrade many of these chemicals (see Appendix D, Testing Methods in Biodegradation).

The chemical structure of a contaminant will affect its biodegradation in two ways (Hutzinger and Veerkamp, 1981). First, the molecule may contain groups or substituents that cannot react with available or inducible enzymes (i.e., these chemical bonds cannot be broken). Secondly, the structure may determine the compound to be in a physical state (adsorbed, gas-phase) where microbial degradation does not easily occur. This seems to be a problem with many of the lipophilic compounds, which have very low solubilities in water.

Generally, the larger and more complex the structure of a hydrocarbon, the more slowly it is oxidized. This may depend upon the type of organism involved and the medium in which it was developed (Texas Research Institute, Inc., 1982). Conflicting conclusions have been reported on this subject. Some authors have proposed that aliphatic, long-chain molecules are attacked more readily than short chains, with hydrocarbons in the range of $C_{10}H_{22}$ to $C_{16}H_{34}$ being oxidized by soil bacteria more readily than those of lower weight. Saturated compounds, e.g., n-alkanes, are highly degraded, while asphaltenes and aromatics are often resistant to microbial attack (Jobson, Cook, and Westlake, 1972). Other workers have suggested that although n-alkanes are probably metabolized more rapidly than naphthenes or other aromatics, the reactions appear to be slower with increasing chain length (possibly because of differences in water solubility, and that the n-alkanes with shorter chains (from C_5 to C_9) are more easily used as a source of carbon and energy by

microorganisms than those with longer chain lengths (from C_{10} to C_{14}) (Williams, Cumins, Gardener, Palmier, and Rubidge, 1981). These undergo oxidation to form alcohols, aldehydes, and acids. The most degradable alkanes are those with molecular weights in the C_6 to C_{28} range (Perry, 1968). Aromatic compounds are the least degradable by microbes, and straight-chain paraffins are the easiest to degrade (Evans, Deuel, and Brown, 1980).

The degree of substitution affects biodegradation. Compounds that possess amine, methoxy, and sulfonate groups, ether linkages, halogens, branched carbon chains, and substitutions at the meta position of benzene rings are generally persistent (Knox, Canter, Kincannon, Stover, and Ward, 1968). Addition of aliphatic sidechains increases the susceptibility of cyclic hydrocarbons to microbial attack (Atlas, 1978c). Linear nonbranched compounds are more easily biodegraded than are branched forms and rings (Pettyjohn and Hounslow, 1983). The side chains of the latter are generally attacked first. Changes in n-alkane to isoprenoid hydrocarbon ratios occur in oil spills as biodegradation proceeds (Haines Pesek, Roubal, Bronner, and Atlas, 1981). Phenanthrenes and dibenzothiophenes with C_2 and greater substitution are relatively resistant to biodegradation, while unsubstituted and C_1 substituted 2- and 3-ring condensed aromatics are subject to abiotic and biotic losses. The number and locations of fused rings in polynuclear aromatics are important in determining the rates of their decomposition (Sims and Overcash, 1983). Hydrocarbons that are strong fat solvents may be less readily tolerated or assimilated than those that are less likely to dissolve cell lipids (ZoBell, 1946).

Table A.2-14 summarizes organic groups subject to microbial metabolism by aerobic respiration, anaerobic respiration, and fermentation (Environmental Protection Agency, 1985b). Oxidation indicates that the compound is used as a primary substrate, and cooxidation indicates that the compound is cometabolized. These tables provide only a general indication of degradability of compounds, and treatability studies will usually be required to determine the degradability of specific waste components.

Several linkages may be readily susceptible to biodegradation (Kearney and Plimmer, 1970):

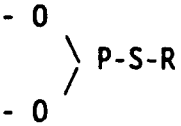
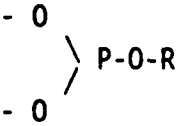
1. $R\text{-}NH\text{-}CO_2R'$
2. $R\text{-}NH\text{-}COR'$
3. 
4. 
5. $R\text{-}CHCl\text{-}COO\text{-}$
6. $R\text{-}CCl_2\text{-}COO\text{-}$

Table A.2-14. Summary of Organic Groups Subject to Biodegradation (Environmental Protection Agency, 1985b)

Substrate Compounds	Respiration		Fermentation	Oxidation	Cooxidation
	Aerobic	Anaerobic			
Straight Chain Alkanes	+	+	+	+	+
Branched Alkanes	+	+	+	+	+
Alcohols	+	+		+	
Aldehydes, Ketones	+	+		+	
Carboxylic Acids	+	+		+	
Cyclic Alkanes	+		+	+	+
Unhalogenated Aromatics	+	+		+	+
Phenols	+	+	+	+	+
Fused Ring Hydroxy Compounds	+				
Phenols - Dihydrides, Polyhydrides	+			+	+
Two- and three-ring Fused Polycyclic Hydrocarbons	+			+	

Aliphatic acids, anilides, carbamates, and phosphates are generally degraded within a short time in the soil (Kaufman, 1983). The rate at which linkages are hydrolyzed will depend upon the nature of R and R'. Chemicals that degrade with an initial ester hydrolysis reaction are relatively short-lived in soil, whereas those that initially undergo dealkylation tend to be somewhat more persistent (Kaufman and Plimmer, 1972). Chemicals that are initially dehalogenated are variable in their persistence. Halogenated aliphatic acids are readily degraded, whereas, halogenated benzoic acids and s-triazines are intermediate in their persistence. Table A.2-15 lists some of the major chemical classes and their relative persistence and initial degradation reactions (Kaufman, 1983).

An inverse relationship and a high correlation between microbial transformation rates and van der Waal's radius of eight phenols have been found, suggesting the latter as useful for predicting degradability of xenobiotics (Paris, Wofe, Steen, and Baughman, 1983).

2.2.1.9 Environmental Factors

Metabolism by indigenous microflora is influenced by environmental factors, such as light, temperature, pH, presence of cometabolites, reactive radicals, other organic and inorganic compounds, and available oxygen, nitrogen, and phosphorus, as well as the physical state of the oil (Cooney, Silver, and Beck, 1985). The environment influences biodegradation by regulating both the bioavailability of the compound and the activity of the degraders. Salinity, temperature, chlorophyll, nitrogen, and phosphorus concentrations have been correlated with rates of biodegradation in surface water environments. The numbers of hydrocarbon-using organisms may also be enhanced by prior pollution of a site.

2.2.1.10 Toxicity

Organic compounds may not be readily degraded in groundwater when the microbial population is low, the nutrient balance is inadequate, or because of toxicity from contaminant overloading (Pettyjohn and Hounslow, 1983). Crude oils are mixtures of tremendous complexity, containing hundreds of hydrocarbon and nonhydrocarbon components, many of them still unidentified and some of these very toxic toward microorganisms (Atlas and Bartha, 1973b). Some compounds may be more toxic to microbes than others (Scholze, Wu, Smith, Bandy, and Basilico, 1986), and the presence of inhibitory substances in oil can delay or prevent the biodegradation of otherwise suitable hydrocarbon substrates (Bartha and Atlas, 1977).

When the structural features necessary for toxicity are compared with those features permitting degradation in the environment for target organisms, differences are found among the various chemical classes (Kaufman and Plimmer, 1972). In some classes, those structural features contributing to toxicity are coincident with those necessary for degradability; in other chemical classes they are diametrical. In all classes, however, the relationships are mediated by substituent type, number, and position.

The structure-toxicity and structure-degradability relationships of certain halogenated aliphatic acids are quite similar; i.e., the most phytotoxic structures were also the most readily degradable (Kaufman, 1983). Meta-

Table A.2-15. Relative Persistence and Initial Degradative Reactions of Nine Major Organic Chemical Classes (Panchal and Zajic, 1978)

Chemical Class	Persistence	Initial Degradative Process
Carbamates	2 to 8 weeks	Ester hydrolysis
Aliphatic acids	3 to 10 weeks	Dehalogenation
Nitriles	4 months	Reduction
Phenoxyalkanoates	1 to 5 months	Dealkylation, ring hydroxylation or oxidation
Toluidine	6 months	Dealkylation (aerobic) or reduction (anaerobic)
Amides	2 to 10 months	Dealkylation
Benzoic acids	3 to 12 months	Dehalogenation or decarboxylation
Ureas	4 to 10 months	Dealkylation
Triazines	3 to 18 months	Dealkylation or dehalogenation

substitution (*para* to a free *ortho* position) confers resistance to biodegradation and eliminates phytotoxic activity. Halogenation in the *para* position increases both phytotoxicity and biodegradability of phenoxyacetates. Increasing the length of the side chain affects both phytotoxicity and biodegradability.

Methyl-, dimethyl-, and trimethyl-naphthalenes are more toxic than naphthalene to the freshwater alga, *Selenastrum capricornatum*, while dibenzofuran, fluorene, phenanthrene, and dibenzothiophene are even more toxic to this organism (Hsieh, Tomson, and Ward, 1980). In general, compounds with higher boiling points are more toxic.

C_2 to C_6 alkanes are inhibitory to some microorganisms possibly because their size allows them to penetrate into cell membranes (Hornick, Fisher, and Paolini, 1983). This is also seen with cycloalkanes of similar size and could be the reason for the "toxicity" of short chain alkanes seen with a few microorganisms.

The toxicity of PAHs to microorganisms is also related to their water solubility (Sims and Overcash, 1983). Aromatic hydrocarbons in water-soluble fractions of petroleum products are toxic to aquatic organisms, but rapid volatilization of low molecular weight hydrocarbons limits the exposure time (Coffey, Ward, and King, 1977). The vapor phase of short chain alkanes is less toxic than the liquid phase. The toxicity of the short chain alkanes is also related to temperature, since a higher temperature will increase the amount of alkane in the vapor phase and decrease the concentration of the liquid alkane. In cold water, however, these compounds may delay the onset of biodegradation for several weeks (Bartha and Atlas, 1977).

While low molecular weight aromatic hydrocarbons are quite toxic to microorganisms, they can be metabolized when present in low concentrations. Condensed polyaromatic hydrocarbons are less toxic to microorganisms but are metabolized only rarely and at slow rates. Cycloalkanes are highly toxic and serve as growth substrates for isolated organisms only in exceptional cases. Some are readily degraded, however, by the cometabolic attack of mixed microbial communities. In general, the biotransformation process for PAHs with more than three rings appears to be cometabolism (Sims and Overcash, 1983).

Low molecular weight hydrocarbons solvate and, hence, destroy the lipid-containing pericellular and intracellular membrane structures (Bartha and Atlas, 1977). Liquid hydrocarbons of the *n*-alkane, *iso*-alkane, cycloalkane, and aromatic type with carbon numbers under 10 all share this property to varying degrees.

Floating oil is able to concentrate hydrophobic pollutants (Bartha and Atlas, 1977). This makes the material more toxic and interferes with microbial degradation.

In certain situations, it may be possible to modify the chemical composition of oil, rendering it more susceptible to biodegradation (Atlas, 1977). The toxic components in the low molecular weight and low boiling range can be removed by temporary heating, ignition, and burning of the oil, and artificially increasing air movement over the oil (Atlas and Bartha, 1972a; Atlas, 1975). Oil is particularly difficult to ignite in many aquatic environments (Fay,

1969). Burning would probably remove toxic components and many other hydrocarbons. However, burning of the substance is not without problems, and, depending upon the chemical composition of the oil, may create a residual that is more resistant to biodegradation.

2.2.1.11 Concentration

The chemical concentration may also affect the level of tolerance (see Section 2.2.2) (Scholze, Wu, Smith, Bandy, and Basilico, 1986). The term "xenobiotic compound" refers not only to compounds with structural features foreign to life but also to those compounds that are released in the environment by the action of man and, thereby, occur in a concentration that is higher than natural (Leisinger, 19830). The concentration of hydrocarbons in water has two effects (Texas Research Institute, Inc., 1982). At low concentrations, all fractions are likely to be attacked, but at high concentrations, only those fractions most susceptible to degradation will be attacked. Also, if the hydrocarbon mixture contains water-soluble toxic substances, their effect is intensified at high concentrations. See also Section 2.2.3.

2.2.1.12 Naturally Occurring Organic Materials

Naturally occurring organic materials can influence the ability of microorganisms to degrade pollutants (Shimp and Pfaender, 1984). After adaptation of a microbial community to four types of compounds, it was found that amino acids, fatty acids and carbohydrates stimulated biodegradation of monosubstituted phenols, while humics decreased biodegradation rates. Many aromatic compounds bind to particulate material and, thus, eventually reside in sediments of natural aquatic ecosystems (Horowitz and Tiedje, 1980).

2.2.1.13 Abiotic Hydrolysis and Oxidation

The effects of these processes on particular contaminants in the groundwater zone are unknown (Mackay, Roberts, and Cherry, 1985). It is believed, however, that most chemical reactions in the groundwater are likely to be slow in comparison with transformations mediated by microorganisms (Cherry, Gillham, and Barker, 1984).

2.2.1.14 Biological Factors

Increased persistence of chemicals may result from several types of biological interactions: 1) the biocidal properties of the chemicals to soil microorganisms may preclude their biodegradation, 2) direct inhibition of the adaptive enzymes of effective soil microorganisms, and 3) inhibition of the proliferation processes of effective microorganisms (Kaufman, 1983). Inhibition of microbial degradation may ultimately affect mobility of a chemical in soil.

It should be realized that biodegradability of a petroleum compound is a result of the action of the mixed flora present at the location, and that the material being degraded is actually a complex mixture of hydrocarbons, some of which contribute to the breakdown of others (Cooney, Silver, and Beck, 1985). It is also possible that problems could arise involving the degradation, persistence, or toxicity of organic chemicals when several wastes or their residues are present in the soil together (Kaufman, 1983). These factors should be

taken into account when assessing the microbiological potential for petroleum degradation.

It has been proposed that the observed recalcitrance of many compounds *in vitro* may be due to the lack of properly designed experiments under the appropriate conditions that are conducive to degradation (Hegeman, 1972). Recalcitrance could also be due to insufficient time to evolve enzymatic pathways to degrade certain chemicals. The acclimation or induction of enzymes that catalyze the necessary reactions in the microbial population is an important factor determining biodegradability (Paris, Wolfe, Steen, and Baughman, 1983).

2.2.2 Rate of Biodegradation

When the biodegradability or composition of a waste constituent is unknown, it is prudent to undertake a laboratory investigation of the kinetics of biodegradation of the material (Thibault and Elliott, 1979). Respirometric techniques have been used to establish biodegradation rates (kinetics), the potential for inhibition of these rates at various waste concentrations, oxygen and nutrient requirements, and temperature effects.

In many instances, it is possible that the minimum set of factors or variables (at least for substrates that are mineralized) that need to be considered in assessing the rate of degradation of a compound are the concentration of the compound and the abundance and activity of organisms capable of mineralizing the compound (Alexander, 1986). For instance, studies indicate that the rate of mineralization of naphthalene is determined primarily by the presence of elevated hydrocarbon-degrading microbial populations and may not be directly related to elevated populations of heterotrophic bacteria or sediment organic carbon content (Heitkamp, Freeman, and Cerniglia, 1987). At 1 mg/l tertiary butyl alcohol, it appears the microbial population receives insufficient energy to cause a population increase and utilization rates remain slow (Novak, Goldsmith, Benoit, and O'Brien, 1985). Rates are faster at higher concentrations, where growth can be better supported. Environmental factors can also affect the rate of biodegradation. For example, the action of a *Nocardia* sp. on hexadecane suggested that the rate of natural biodegradation of oil in marine environments was limited by low temperatures and phosphorus concentration, but not by the concentrations of naturally occurring nitrogen (Mulkins-Phillips and Stewart, 1974b).

An approach has been developed to measure rates of degradation based upon the examination of metabolic kinetics using radiolabeled substrates (Pfaender and Klump, 1981). The measurements require short incubations (8 to 10 hr), which should yield rates close to those occurring in nature. The method has been applied to fresh water, estuarine, and oceanic environments. There is a fivefold decrease in rates of metabolism from fresh to estuarine water and a tenfold further decrease from estuarine to ocean water.

Very rapid mineralization rates (e.g., for naphthalene) have been reported for some sediments that are chronically exposed to very high concentrations of degradable hydrocarbons (Heitkamp, Freeman, and Cerniglia, 1987). The mineralization rate and half-life calculated for naphthalene was about 2.9 percent/day and 2.4 weeks (17 days), respectively, for such a source; while the half-life was 4.4 weeks with sediment from a pristine environment.

Mineralization of glucose by Salmonella at ng/ml levels stopped, and cell death occurred before the substrate was exhausted, suggesting a significant role for maintenance energy in determining the kinetics of mineralization of organic chemicals at low concentrations (Simkins, Schmidt, and Alexander, 1984).

Microorganisms have been found to be able to degrade one- and two-ringed aromatic hydrocarbons with high reaction rates down to extremely low concentration levels (i.e., <1 ug/l), given sufficient oxygen and nutrients (Gray, 1978). It is estimated that polluting oil in the sea might be biodegraded at rates as high as 100 to 960 $mg/m^3/day$ (Bartha and Atlas, 1977). Degradation rates for hexadecane have been measured to be 0.050 g (Knetting and Zajic, 1972) and 0.015 $g/m^3/day$ (Knetting and Zajic, 1972; Seki, 1976) at summer temperatures, 0.001 $g/m^3/day$ in the colder waters of Alaska, and a rate even lower than this in the open waters of the Arctic Ocean (Robertson, Arhelger, Kinney, and Button, 1973).

While microbes are important in degrading many petroleum hydrocarbons in ocean waters, the low degradation rates for fluorene and benzopyrene suggest that this does not hold for high molecular weight aromatics (Lee and Ryan, 1976). Biodegradation of polynuclear aromatics appears to be inversely correlated to molecular size and ring condensation (Bossert, 1983). PAHs are degraded at much lower rates than mono- and dinuclear aromatics and n-paraffins in ocean waters (Bartha and Atlas, 1977). Turnover times of the order of months are found for paraffins and light aromatics and of the order of several years for polynuclear aromatics. Degradation rates are high in spring and low in winter.

Naphthalene had the highest degradation rate of the relatively nonvolatile hydrocarbons tested, followed in decreasing order by methylnaphthalene, heptadecane, hexadecane, octadecane, fluorene, and benzopyrene (Thomas and Alexander, 1983). The degradation rate of all hydrocarbons was higher in the estuarine area than in water from offshore. The calculated turnover times (time required to convert all hydrocarbon to carbon dioxide) are presented in Table A.2-16 (Science Applications International Corporation, 1985a). Relatively little is known of the kinetics of degradation of mixed substrates at low concentrations, or the possible interactions among primary and secondary substrates and bacteria (McCarty, Reinhard, and Rittmann, 1981). The presence of additional substrates in the soil or lake water may also alter the kinetics of mineralization of low concentrations of organic pollutants (Schmidt, Scow, and Alexander, 1985). With a pure culture of Pseudomonas acidovorans, acetate and phenol disappeared at an equal rate, when they were at low concentrations. However, phenol mineralization was repressed at high acetate concentrations.

2.2.3 Effect of Hydrocarbon Concentrations on Degradation

Table A.2-17 lists concentrations at which certain compounds have been found to be toxic in industrial waste treatment (Environmental Protection Agency, 1985b). Microorganisms present in the subsurface, however, may be more tolerant to high concentrations of these compounds. This must be determined on a case-by-case basis.

The concentration of a contaminant will affect the number of organisms present. It has been noted that higher concentrations of gasoline in

Table A.2-16. Turnover Times for Microbial Hydrocarbon Degradation in Coastal Waters (Lee and Ryan, 1976)

Compound	Concentration (ppb)	Date Locality	Turnover time (days)
Benzpyrene	5	Feb--Sk	0
	5	May--Sk	3,500
	5	May--O	0
Fluorene	30	Feb--Sk	0
	30	Feb--O	0
	30	May--Sk	0
	30	June--Sk	1,000
Heptadecane	8	May--Sk	80
	15	May--Sk	60
	30	May--Sk	54
	30	May--Sk	170
Hexadecane	25	Feb--Sk	500
	25	April--Sk	210
	25	April--O	1,000
Naphthalene	40	Feb--Sk	500
	40	May--Sk	46
	40	May--Sk	79
	130	May--Sk	30
	130	May--O	330
Methyl-naphthalene	40	Feb--Sk	500
	40	May--Sk	50
Octadecane	16	May--Sk	100
Toluene	20	May--Sk	17
	20	May--Sk	17
	20	May--O	40

Sk = Skidaway River (3m)
O = offshore water (10m)

Table A.2-17. Problem Concentrations of Selected Chemicals (Environmental Protection Agency, 1985b)

Chemical	Problem Concentration (mg/l)	
	Substrate Limiting[a]	Nonsubstrate Limiting[b]
Formaldehyde	--	50 - 100
Acetone	--	>1000
Phenol	>1000	300 - 1000
Ethyl benzene	>1000	--
Dodecane	>1000	--

a = Substrate limiting represents the condition in which the subject compound is the sole carbon and energy source.

b = Nonsubstrate limiting represents the condition in which other carbon and energy sources are present.

contaminated water were related to higher counts of microorganisms (McKee, Laverty, and Hertel, 1972; Litchfield and Clark, 1973). Waters containing less than 10 ppm of hydrocarbon had populations of bacteria less than 10^3, while concentrations of hydrocarbon in excess of 10 ppm sometimes supported growth of 10^6 bacteria/ml.

It has been shown that the persistence of a compound increases as the initial concentration increases (Hamaker, 1972). The reduced rate is explained either by the limited active sites available (Hance and McKone, 1971), or by a toxic effect on microorganisms or enzyme inhibition (Hurle and Walker, 1980). With PAHs, there is an increasing trend of initial rate of degradation as the initial concentration increases (Sims and Overcash, 1983). Table A.2-18 summarizes rates of degradation from the literature.

2.2.3.1 Examples of Degradable Levels of Organic Compounds

Examples of concentrations of various compounds that have been shown to be tolerated and degraded are discussed below and summarized in Table A.2-19.

* Biological degradation is the generally recognized treatment method for phenolic wastewater containing phenol concentrations up to 500 ppm (Roberts, Koff, and Karr, 1988). At phenol concentrations of 10,000 ppm, biodegradation of 99 to 100 percent have been reported for phenol, methylphenols, nitrophenols, and chlorophenols.

* Aeromonas, Alcaligenes, Pseudomonas, and Vibrio were found to degrade South Louisiana Crude Oil and motor oil at concentrations of 1.0 and 5.0 percent, but growth on 0.01 percent was very limited (Frieze and Oujesky, 1983).

* Approximately 30,000 gal of gasoline contaminated over 75,000 ft^2 (Brown, Norris, and Brubaker, 1985). Five pumping wells recovered 18,500 gal, and bioreclamation was initiated to remediate the soil and groundwater. Nutrients were periodically injected and air was continuously sparged over 10 months through 14 wells, and the gasoline level in the soil was reduced to less than 50 ppm.

* From 700 to 1,400 gal of mixed fuels/solvents (45 percent aromatics/55 percent alkanes) were confined to a tank vault (Brown, Loper, and McGarvey, 1985). Free product recovery of about 700 gal was followed by enhanced bioreclamation and ended with carbon treatment. Groundwater levels during bioreclamation dropped from 22 to 45 ppm to <550 ppb in 2 1/2 months.

* After nine months of treatment of soil contaminated with gasoline, soils in a highly contaminated tank storage area still showed signs of gasoline contamination at levels of 500 to 100 ppm and the average concentrations of dissolved carbon (DOC) was 20 ppm (Minugh, Patry, Keech, and Leek, 1983). After the biostimulation program ended, gasoline odors and a cloudy sheen were detected in some of the pits. However, samples showed continued improvement. The DOC in the water had fallen to the point where 71 percent of the measurements fell below 5 ppm and 50 percent were under 2 ppm. Since the DOC levels initially were not given, it is difficult to evaluate the efficiency of the cleanup.

Table A.2-18. Kinetic Parameters Describing Rates of Degradation of Aromatic Compounds in Soil Systems (Sims and Overcash, 1983)

PAH	Initial Concentration (ug/g soil)	k (day^{-1})	Rate of Transformation (ug/g-day)	$t_{1/2}$[a] (days)	
Pyrocatechol	500	3.47	1,735	0.2	m
Phenol	500	0.693	364.5	1.0	m
Phenol	500	0.315	157.5	2.2	l
Fluorene	0.9	0.018	0.016	39	m
Fluorene	500	0.347	173.3	2	m
Indole	500	0.693	364.5	1.0	m
Indole	500	0.315	157.5	2.2	l
Naphthol	500	0.770	385	0.9	m
Naphthalene	7.0	5.78	40.4	0.12	m
Naphthalene	7.0	0.005	0.035	125	l
Naphthalene	25,000	0.173	4,331	4	h
1,4-Naphthoquinone	500	0.578	288.8	1.2	m
Acenaphthene	500	0.173	86.6	4	m
Acenaphthene	5	2.81	22.6	0.3	m
Anthracene	3.4	0.21	0.714	3.3	l
Anthracene	13.7	0.004	0.054	175	m
Anthracene	10.3	0.005	0.050	143	m
Anthracene	11.4	0.006	0.073	108	m
Anthracene	40.0	0.005	0.208	138	m
Anthracene	36.4	0.005	0.196	129	m
Anthracene	25,000	0.198	4,950	3.5	h
Phenanthrene	2.1	0.027	0.056	26	m
Phenanthrene	25,000	0.277	6,930	2.5	h
Carbazole	500	0.067	33	10.5	m
Carbazole	5	0.231	1.16	3	m
Benz(a)anthracene	0.12	0.046	0.005	15.2	l
Benz(a)anthracene	0.12	0.0001	0.00001	6,250	m
Benz(a)anthracene	3.5	0.007	0.024	102	m
Benz(a)anthracene	20.8	0.003	0.062	231	m
Benz(a)anthracene	25.8	0.005	0.134	133	m
Benz(a)anthracene	17.2	0.008	0.060	199	m
Benz(a)anthracene	22.1	0.006	0.130	118	m
Benz(a)anthracene	42.6	0.003	0.118	252	m
Benz(a)anthracene	72.8	0.004	0.257	196	m
Benz(a)anthracene	25,000	0.173	4,331	4	h
Fluoranthene	3.9	0.016	0.061	44	m
Fluoranthene	18.8	0.004	0.072	182	m
Fluoranthene	23.0	0.007	0.152	105	m
Fluoranthene	16.5	0.005	0.080	143	m
Fluoranthene	20.9	0.006	0.125	109	m
Fluoranthene	44.5	0.004	0.176	175	m
Fluoranthene	72.8	0.005	0.379	133	m
Pyrene	3.1	0.020	0.061	35	m

Table A.2-18. Kinetic Parameters Describing Rates of Degradation of Aromatic Compounds in Soil Systems (Sims and Overcash, 1983) (Continued)

PAH	Initial Concentration (ug/g soil)	k (day^{-1})	Rate of Transformation (ug/g-day)	$t_{1/2}$[a] (days)	
Pyrene	500	0.067	33	10.5	m
Pyrene	5	0.231	1.16	3	m
Chrysene	4.4	0	0	-	
Chrysene	500	0.067	33	10.5	m
Chrysene	5	0.126	0.63	5.5	m
Benz(a)pyrene	0.048	0.014	0.007	50	l
Benz(a)pyrene	0.01	0.001	0.00001	694	l
Benz(a)pyrene	3.4	0.012	0.041	57	m
Benz(a)pyrene	9.5	0.002	0.022	294	m
Benz(a)pyrene	12.3	0.005	0.058	147	m
Benz(a)pyrene	7.6	0.003	0.020	264	m
Benz(a)pyrene	18.5	0.023	0.312	30	m
Benz(a)pyrene	17.0	0.002	0.028	420	m
Benz(a)pyrene	32.6	0.004	0.129	175	m
Benz(a)pyrene	1.0	0.347	0.347	2	h
Benz(a)pyrene	0.515	0.347	0.179	2	h
Benz(a)pyrene	0.00135	0.139	0.0002	5	h
Benz(a)pyrene	0.0094	0.002	0.00002	406	l
Benz(a)pyrene	0.545	0.011	0.006	66	l
Benz(a)pyrene	28.5	0.019	0.533	37	l
Benz(a)pyrene	29.2	0	0	-	
Benz(a)pyrene	9,100	0.018	161.7	39	h
Benz(a)pyrene	19.5	0.099	1.93	7	h
Benz(a)pyrene	19.5	0.139	2.70	5	h
Benz(a)pyrene	19.5	0.231	4.50	3	h
Benz(a)pyrene	130.6	0.173	22.63	4	h
Benz(a)pyrene	130.6	0.116	15.08	6	h
Dibenz(a,h)anthracene	9,700	0.033	320.1	21	h
Dibenz(a,h)anthracene	25,000	0.039	962.5	18	h

[a] l = low temperature range (<15°C)
m = medium temperature range (15 to 25°C)
h = high temperature range (>25°C)

Table A.2-19. Initial and Final Concentrations of Compounds Susceptible to Biodegradation

Compound	Time for Degradation	Initial Concentration	Final Concentration	Organism/ Source
South Louisiana Crude Oil and Motor Oil[a]		1.0%, 5.0%		Aeromonas, Alcaligenes, Pseudomonas, Vibrio
Mixed Fuels/ Solvents[b]	2 1/2 mo	22 to 45 ppm (Groundwater)	<550 ppb	
Gasoline[c]		100 to 500 ppm	2 to 5 ppm	
Methylene chloride[d]	1 yr	91 ppm (Groundwater)	<1 ppm	
Acetone[d]	1 yr	54 ppm	<1 ppm	
Acrylonitrile[e]	3 mo	1000 ppm (Groundwater)	1 ppm	Mutant bacteria
Acrylonitrile[f]	1 mo	1000 ppm	lod	Mutant bacteria
Phenol[f]	40 d	31 ppm	30 ppm	Mutant bacteria
Organic chemicals[g]		<1000 ppm (Soil)	<1 ppm	Indigenous and hydro-carbon degrading bacteria
Methylene chloride[h]	2 1/2 mo	2500 mg/l	<100 mg/l	Commercial hydrocarbon degrading bacteria
Dichlorobenzene[h]	2 1/2 mo	800 mg/l	<50 mg/l	"

Table A.2-19. Initial and Final Concentrations of Compounds Susceptible to Biodegradation (Continued)

Compound	Time for Degradation	Initial Concentration	Final Concentration	Organism/ Source
p-Cresol[i]		8 ppm		
Hydrocarbon[j]		10 ppm		
Gasoline[k]	10 mo	11,500 gal/ 75,000 ft^2	<50 ppm	
Gasoline[k]	18 mo another 6 mo	5 to 8 ppm 2.4 ppm (Groundwater)	2.4 ppm <500 ppb	Indigenous organisms
Gasoline[k]	25 mo (air sparging) 10 mo (microbial nutrient)	15 ppm (Groundwater)	2.5 ppm 200 to 1200 ppb	Indigenous organisms
Petroleum distillate[l]	21 d	12,000 ppm	>1 ppm	BI-CHEM-SUS-8
Formaldehyde[l]	22 d	1400 ppm	>1 ppm	PHENOBAC
Phenols[m]	7 hr	1500 ppm	>1 ppm	Azotobacter
Phenols[m]		10,000 ppm	0 to 100 ppm	
Phenol[n]		32 ng/g soil		
Solvent/fuel mixture (aliphatic and aromatic hydrocarbons)[o]	2-1/2 mo	23 ppm	0.5 ppm	Landfarming
Gasoline[o]	10 mo	30 to 40 ppm (groundwater)	>1 ppm	
	10 mo	2,000 to 3,000 ppm (soil)	>50 ppm	
Phenol[p]	7 days 7 days	5 mg/l 10 mg/l	0 mg/l 0 mg/l	Domestic wastewater
Naphthalene[p]	7 days 7 days	5 mg/l 10 mg/l	0 mg/l 0 mg/l	Domestic wastewater
Benzene[p]	7 days	5 mg/l	0 mg/l	Domestic

Table A.2-19. Initial and Final Concentrations of Compounds Susceptible to Biodegradation (Continued)

Compound	Time for Degradation	Initial Concentration	Final Concentration	Organism/ Source
Benzene[p]	14 days	10 mg/l	0 mg/l	wastewater
Toluene[p]	7 days	5 mg/l	0 mg/l	Domestic
	7 days	10 mg/l	0 mg/l	wastewater
Anthracene[p]	21 days	5 mg/l	0.4 mg/l	Domestic
	21 days	10 mg/l	5 mg/l	wastewater
Phenanthrene[p]	7 days	5 mg/l	0 mg/l	Domestic
	7 days	10 mg/l	0 mg/l	wastewater
1,2-Benzanthracene[p]	7 days	5 mg/l	3 mg/l	Domestic
	7 days	10 mg/l	6 mg/l	wastewater
Pyrene[p]	7 days	5 mg/l	0 mg/l	Domestic
	21 days	10 mg/l	10 mg/l	wastewater
Methanol[q]	>30 days	100 mg/l	<lod	Soil
	>200 days	1000 mg/l	<lod	(aerobic and anaerobic)
Tertiary butyl alcohol[q]	>1 mo	10 mg/l	<lod	Soil
	>1 yr	70 mg/l	<lod	
m-Xylene[r]		0.4 mM		Denitrifying bacteria
Aliphatic and aromatic hydrocarbons (fuels/solvents)[s]	2 1/2 mo	23 ppm	0.05 ppm	
Gasoline[s]	10 mo	30 to 40 ppm	<1 ppm	
Formaldehyde[t]	24 d	>700 ppm	1 ppm	Hydrobac[tm]
Toluene[u]	100 d	10,329 ppm (Groundwater)	>10 ppb	

lod = limits of detection = 50 ppb

Table A.2-19. References:

a = (Frieze and Oujesky, 1983)
b = (Brown, Loper, and McGarvey, 1985)
c = (Minugh, Patry, Keech, and Leek, 1983)
d = (Jhaveria and Mazzacca, 1982)
e = (Polybac Corporation, 1983)
f = (Walton and Dobbs, 1980)
g = (Ohneck and Gardner, 1982)
h = (Quince and Gardner, 1982)
i = (Pritchard, Van Veld, and Cooper, 1981)
j = (Ehrlich, Schroeder, and Martin, 1985)
k = (Brown, Norris, and Brubaker, 1985)
l = (Environmental Protection Agency, 1985b)
m = (Roberts, Koff, and Karr, 1988)
n = (Scow, Simkins, and Alexander, 1986)
o = (Niaki, Pollock, Medlin, Shealy, and Broscious, Draft)
p = (Tabak, Quave, Mashni, and Barth, 1981)
q = (Novak, Goldsmith, Benoit, and O'Brien, 1985)
r = (Zeyer, Kuhn, and Schwarzenbach, 1986)
s = (Brown, Longfield, Norris, and Wolfe, 1985)
t = (Sikes, 1984)

* The contaminant levels in a pumping wells declined from an average of 91 ppm methylene chloride and 54 ppm acetone to less than 1 ppm within a year of treatment (Jhaveria and Mazzacca, 1982). The COD of the groundwater from the pumping well was significantly reduced, also. The area near the pumping wells was the last to be treated and still showed fairly high levels of contamination (up to 40 mg/l of methylene chloride), but the monitoring wells closer to the reinjection system had very low levels of methylene chloride; a reduction from about 70 ppm to less than 0.02 ppm was noted for two of the monitoring wells.

* An acrylonitrile spill contaminated the soil and groundwater of a site in Indiana (Polybac Corporation, 1983). Treatment was by pumping groundwater from several wells to a biotreator seeded with mutant bacteria from Polybac and then injection into the groundwater table. The concentrations of acrylonitrile fell from 1000 to 1 ppm within three months. The report did not contain sufficient details to judge the importance of microbial activity in the removal of the acrylonitrile.

* Groundwater contaminated by 7,000 gal of acrylonitrile was treated by air stripping the recovered groundwater and, after concentrations had been reduced enough to allow microbial growth, by adding mutant bacteria (Walton and Dobbs, 1980). The degradation of acrylonitrile occurred rapidly, as the levels fell from 1,000 ppm to the limits of detection within a month. No data were presented that conclusively linked the drop in the concentration of acrylonitrile to activity by the mutant bacteria.

* An accidental spill of 130,000 gal of organic chemicals entered a 15-ft-thick shallow unconfined aquifer and produced contaminant levels as high as 10,000 ppm (Ohneck and Gardner, 1982). An investigation into stimulating microbial activity showed that the contaminants were biodegradable at concentrations below 1,000 ppm. Both the indigenous microflora and a specific facultative hydrocarbon degrader were able to biodegrade the materials in a soil/water matrix rapidly when supplied with additional nutrients. The biological treatment process accelerated the removal of the compounds, as shown by a series of soil borings during the treatment process, and reduced the levels of contaminants in the groundwater to less than 1 ppm. The specific hydrocarbon degraders did not increase degradation in laboratory tests beyond that of the native microbes and may not have significantly increased <u>in situ</u> biodegradation. No data were presented that showed that the number of the specific hydrocarbon degraders increased in the subsurface or that they were able to outcompete the indigenous microflora in degrading contaminants.

* Dichlorobenzene, methylene chloride, and trichloroethane contaminated groundwater at levels up to 2,500 mg/l (Quince and Gardner, 1982). The treated water was inoculated with commercial hydrocarbon-degrading bacteria and nutrients injected into the subsurface. The levels of bacteria increased until optimum conditions were established in the reactor and then were injected into the soil. In 2 1/2 months, the levels of methylene chloride fell from 2,500 mg/l to less that 100 mg/l, and the levels of dichlorobenzene fell from 800 mg/l to less than 50 mg/l in a monitoring well. The inoculated bacteria were expected to continue to degrade the contaminants beyond the 95 percent reduction reached before the treatment was terminated. The importance of microbial activity could not be determined from the data presented.

* Soil and rail ballast contaminated with 20,000 gal of 40 percent formaldehyde initially showed a concentration of leachate with greater than 700 ppm formaldehyde (Sikes, 1984). Microorganisms and nutrients were sprayed over the contaminated area. Within 24 days of operation, the concentration had dropped to 1 ppm.

* An artificial outdoor stream channel was dosed with 8 ppm p-cresol for a period of 48 hr, after which time the concentration of the compound decreased rapidly (Pritchard, Van Veld, and Cooper, 1981).

* As toluene levels fell from 10,329 ppb to less than 10 ppb in about 100 days, benzene levels rose, possibly due to demethoxylation of the toluene to benzene (Wilson, Bledsoe, Armstrong, and Sammons, 1986). Then levels of both compounds fell.

2.2.3.2 Low Concentrations

There appears to be a minimum concentration to which a single organic material can be decomposed under steady-state conditions (McCarty, Reinhard, and Rittmann, 1981). Trace organic materials may be biodegradable, but are often below this minimum concentration. In general, biodegradation of such materials can occur only if they are used as secondary substrates; i.e., if there is an abundant primary organic substrate available and bacteria capable of decomposing both. An alternative is decomposition within a nonsteady-state system that has a sufficiently large population of bacteria previously grown on a primary substrate. Biodecomposition is also possible, if several organic substrates are present in a sufficiently large total concentration.

Low levels, or trace concentrations, of some organic substrates would be less than 100 ng/ml, which is characteristic of the concentrations of pollutants in many fresh, estuarine, and marine waters (Alexander, 1986). This level of contamination is also important, since criteria and standards for water quality refer to maximum acceptable levels of many organic pollutants that are below 100 ng/ml (Patrick and Mahapatra, 1968) and since numerous toxicants are harmful at levels in the parts-per-billion range (Batterton, Winters, and van Baalen, 1978).

Microorganisms might not assimilate carbon from chemicals present in trace amounts in natural environments (Alexander, 1985). They might not grow or produce the large, acclimated populations needed for enhanced biodegradation. It is possible to predict the minimum concentration of a chemical necessary to support microbial growth. However, erroneous conclusions may be reached, if data from laboratory studies of chemicals at high concentrations are extrapolated to environments in which the chemicals exist at low concentrations (Alexander, 1986). It is important to use concentrations characteristic of those in nature for laboratory investigations. It is also possible for a chemical to be mineralized at one concentration and cometabolized at another.

Several anomalies have been detected in biodegradation of low concentrations of organic compounds (Alexander, 1986).

1. The rate of mineralization may be less than anticipated if it is assumed that the rates are linearly related to concentration.

2. Chemicals mineralized at one concentration may not be converted to carbon dioxide at lower levels.

3. Organic compounds may not be mineralized at low and presumably nontoxic levels in water, but they may be metabolized to carbon dioxide at still lower concentrations.

4. Mineralization may not follow the commonly described kinetics but may proceed in a biphasic manner.

5. The extent of mineralization in samples from a single body of water may vary markedly.

6. Microbial communities may acclimate to mineralize a substrate even though the substrate concentration is below the threshold level to sustain growth.

7. Compounds may be mineralized in some but not all waters.

SECTION 3

INDIGENOUS MICROORGANISMS IN BIODEGRADATION

3.1 MICROORGANISMS IN SOIL

Often, microbes capable of degrading a compound may remain in the soil after the last treatment, and pure cultures capable of degrading many of these chemicals can still be isolated several years later (Kaufman, 1983). Hydrocarbonoclastic organisms can be recovered from the deep subsurface. Many bacteria and yeasts that could catabolize methanol under aerobic or anaerobic conditions and at low and high concentrations are isolated from deep soil (to 100 ft) (Benoit, Novak, Goldsmith, and Chadduck, 1985).

Table A.3-1 lists a number of organisms, other than true bacteria, and the compounds they are able to degrade (Kobayashi and Rittmann, 1982).

Table A.3-2 shows the species or groups of microorganisms that might be useful for treating contamination by specific types of organic compounds and the conditions that would be most favorable for their development (Kobayashi and Rittmann, 1982).

3.1.1 Bacteria

Of 41 cultures isolated from the soil and groundwater at a site contaminated with gasoline from a leaking gas station tank, 17 were considered to be Pseudomonas, four Flavobacterium, 11 Nocardia, and nine unidentified (Raymond, Jamison, and Hudson, 1978). Many of the cultures were composed of very small cells.

Small cells have been produced experimentally as a result of starvation (Novitsky and Morita, 1978). These "ultramicrobacteria" are particularly suited to survival at low nutrient concentrations and low population densities. The cells are less than 0.3 um in diameter, demonstrate slow growth, and do not significantly increase in size when inoculated on only a nutrient-rich agar medium. Biochemical characterization might not be performed on them due to lack of sufficient growth of the organisms. The need for minimal nutrient concentration and a prolonged incubation time appears to be extremely important for recovering ultramicrobacteria (Tabor, Ohwada, and Colwell, 1981).

Cellovibrio spp. occur in the natural environment as small filterable particles that cannot be subcultured or reverted to normal cells (Tuckett and Moore, 1959). Aerobacter spp. have also been found to form starvation-resistant, small cells (Harrison and Lawrence, 1963). Dwarf cells, some less than 0.08 um in diameter, have been observed in soil samples with electron microscopy (Bae, Costa-Robles, and Casida, 1972). In the last case, many of the cells are too small to be observed by light microscopy and possess fine structural differences from normal-sized soil bacteria.

The propensity of organisms to be attached also affects the type of compounds they will attack. For instance, free-living bacteria are responsible for a large proportion of the metabolism of m-cresol and chlorobenzene, while

Table A.3-1. Degradation of Anthropogenic Compounds by Different Groups of Microorganisms (Kobayashi and Rittmann, 1982)

Microorganism	Compound
Cyanobacteria (blue-green algae)	
Microcystis aeruginosa	Benzene, toluene, naphthalene, phenanthrene, pyrene
Algae	
Selanastrum capricornatum	Benzene, toluene, naphthalene, phenanthrene, pyrene
Actinomycetes	
Nocardia spp.	*n*-paraffins: pentane, hexane, heptane, octane, 2-methylbutane, 2-methylpentane, 3-methylheptane, 2,2,4-trimethylpentane, ethylbenzene[a], hexadecane and kerosene (at 2% but not 4%)[b]
Yeasts	
Trichosporon, Pichia Rhodosporidium, Rhodotorula, Debaryomyces, Endomycopsis,	hexadecane and kerosene (at 2% but not 4%)[b]
Candida parapsilosis, C. tropicalis, C. guilliermondii, C. lipolytica, C. maltosa, Debaryomyces hansenii, Trichosporon sp., *Rhodosporidium toruloides*	(naphthalene, biphenyl, benzo(a)pyrene)[c]

a = (Jamison, Raymond, and Hudson, 1976)
b = (Ahearn, Meyers, and Standard, 1971)
c = (Cerniglia and Crow, 1981)

Table A.3-2. Selective Use of Microorganisms for Removal of Different Anthropogenic Compounds (Kobayashi and Rittmann, 1982)

Microorganism	Selective[a] Characteristics	Significance
Fungi		
Yeast	pH<5, ae-mae; high O_2 tension, pH<5 moisture about 50%	Attacks and partially degrades compounds not readily metabolized by other organisms. Wide range of nonspecific enzymes
Mold		
Algae	ae-mae; light: 600 to 700 nm; low carbon flux	Self-sustaining population, light is primary energy source, partially degrades certain complex compounds, photochemical reactions, oxygenates medium during daylight, supports growth of other microbes, effective in bioaccumulation of hydrophobic substances
Cyanobacteria (blue-green algae)	ae-mae, an; light: 600 to 700 nm; low carbon flux	See algae
Bacteria		
Heterotrophs (aerobic)	ae; proper organic substrate, growth factors as required; Eh: 0.45 to 0.2 V	For many compounds degradation is more complete and faster than under anaerobic conditions. High sludge production
Anaerobic (fastidious)	an; Eh: <-0.2 to -0.4 V	Conditions for abiotic or biological reductive dechlorination, certain detoxification reactions not possible under aerobic conditions; no aeration, little sludge produced
Facultative anaerobes	ae, mae-an; Eh: <-0.2 V	No aeration, reductive dechlorination possible

Table A.3-2. Selective Use of Microorganisms for Removal of Different Anthropogenic Compounds (Kobayashi and Rittmann, 1982) (Continued)

Microorganism	Selective[a] Characteristics	Significance
Photosynthetic bacteria		
Purple sulfur	an (light), mae (dark); Eh: 0 to -0.2 V; S^{-2}: 2 to 8 mM, 0.4 to 1 mM; light: 800 to 890 nm at 1000 to 2000 lux, high intensities near limit; low C flux	Self-sustaining population able to use light energy, conditions right for reductive dechlorination, no aeration
Purple nonsulfur	an; Eh: 0 to -0.2 V; light: 800 to 890 nm; low C flux	See purple sulfur bacteria, also nonspecific enzymes
Actinomycetes	ae, moisture: 80 to 87%, temp.: 23 to 28°C, urea as nitrogen source	Universal scavengers with range of complex organic substrates often not used by other microbes
Oligotrophs (from almost any group above)	ae; carbon flux of <1 mg/l/d; favorable attachment sites	Removal of organic contaminants in trace concentrations, many inducible enzymes for multiple substrates

ae = aerobic; mae = Microaerophilic (<0.2 atm oxygen); an = anaerobic
a = Possible characteristics for selection, not growth range

particle-associated organisms are more important in the degradation of NTA (Palumbo, Pfaender, Paerl, Bland, Boyd, and Cooper, 1983).

3.1.1.1 Aerobes

Selected aerobic hydrocarbon-degrading bacterial strains are discussed below.

1. Pseudomonas

Pseudomonas appears to be the most ubiquitous, and able to adapt to many different man-made compounds (Kobayashi and Rittmann, 1982). Pseudomonas spp. are responsible for the degradation of most of the aromatics in gasoline (Jamison, Raymond, and Hudson, 1976), although efficiency in degrading aromatic hydrocarbons can vary among strains (Kobayashi and Rittmann, 1982). At optimal conditions of 20°C and after 20 days, one isolate had degraded 70 percent of the aromatic fraction while the other had utilized only 40 percent (Solanas and Pares, 1984). Degradation of alkylnaphthalenes by this genus was more extensive with the less substituted aromatics. The site of attack appeared to be the ring system rather than the alkyl chains.

P. putida was isolated from aviation fuel and grew rapidly using the jet fuel as the sole source of carbon and energy (Williams, Cumins, Gardener, Palmier, and Rubidge, 1981). It grew on and oxidized a wide range of alkanes, with optimum growth occurring on octane. It was able to oxidize straight chain fatty acids ranging from acetate (C_2) to palmitic acid (C_{16}).

A mutant of P. putida CB-173 (ATCC 31800), which is active at temperatures as low as 1° to 4°C, has been developed by Sybron Corp. for degrading phenolic wastewaters during the winter months (Roberts, Koff, and Karr, 1988).

Pseudomonas spp. were found to grow on aromatic constituents of gasoline as a sole source of carbon (Jamison, Raymond, and Hudson, 1976). Moderate to heavy growth was obtained with some isolates on n-pentane, n-hexane, n-heptane, n-octane, 2-methylbutane, toluene, and m- and p-xylene. Light or questionable growth was observed on 3-methylheptane and 2,2,4-trimethylpentane.

P. oleovorans is being used to convert C_6 to C_{12} alkanes to 1,2-epoxides and n-alkanes first to primary alcohols, then to aldehydes, then to carboxylic acids (Roberts, Koff, and Karr, 1988).

2. Beijerinckia

Strains of this genus are very active in hydrocarbon degradation. After growth on succinate in the presence of biphenyl, a mutant strain oxidized benzo(a)pyrene and benzo(a)anthracene to cis-9,10-dihydroxy-9,10-dihydrobenzo(a)pyrene and cis-1,2-dihydroxy-1,2-dihydrobenzo(a)anthracene, respectively (Gibson, 1976).

3. Acinetobacter

These organisms have been frequently encountered as hydrocarbon degraders. They grow rapidly with cyclohexanol as the sole source of carbon, with attack

of cyclohexane by other organisms being initiated by hydroxylation (Donoghue, Griffin, Norris, and Trudgill, 1976).

4. Actinomycetes

Morphologically intermediate between bacteria and fungi, these organisms (which are considered bacteria) are found in environments in which unusual compounds are encountered (Kobayashi and Rittmann, 1982). They attack a wide variety of complex organic compounds, including phenols, pyridines, glycerides, steroids, chlorinated and nonchlorinated aromatic compounds, paraffins, other long-chain carbon compounds, and lignocellulose.

A common actinomycete in aquatic systems is Nocardia; e.g., N. aramae, which grows under low nutrient conditions (oligotrophically), such as in distilled water. N. globerula grows rapidly with cyclohexanol as the sole source of carbon, with attack of cyclohexane by other organisms being initiated by hydroxylation (Donoghue, Griffin, Norris, and Trudgill, 1976).

Advantages to using these organisms in surface treatment systems are that they produce less sludge than bacteria and fungi, grow over a wide temperature range (from psychrophilic to thermophilic), are resistant to desiccation, and have a wide pH range (Kobayashi and Rittmann, 1982). Nocardia spp. are able to utilize n-paraffins from gasoline as a sole source of carbon (Jamison, Raymond, and hudson, 1976) (see Table A.3-1). Actinomycetes might be especially useful in treatment of contaminated soil where a composting technique would be practical (Kobayashi and Rittmann, 1982).

Organic decomposition by actinomycetes results in metabolites that can be mineralized by other organisms. Therefore, mixed cultures are necessary, if actinomycetes are to be used.

5. Pseudomonas methanica

This organism has the ability to use only methane and methanol for growth (Zajic, 1964). It can also cometabolize ethane, propane, and n-butane.

3.1.1.2 Anaerobes

3.1.1.3 Oligotrophs

3.1.1.4 Counts in Uncontaminated Soil

Direct count in uncontaminated soil ranged from 10^6 to 10^7 organisms/g in the literature, while viable counts were reported from zero to 10^8 CFU/g. Table A.3-3 compares counts in different soil types at different depths. These counts assumed that there are 50 umoles phospholipid/g dry weight of bacteria and that there are 10^{12} bacteria/g (Gehron and White, 1983). However, these estimates may be low since subsurface bacteria are smaller than surface bacteria, due to severe nutrient limitation (Webster, Hampton, Wilson, Ghiorse, and Leach, 1985), and a gram of bacteria may contain many more than 10^{12} organisms/g of soil. Apparently, many of the bacteria in soil environments exist as cells smaller than 1 um in size (Ghiorse and Balkwill, 1985b).

Table A.3-3. Plate Counts versus Soil Depth and Type (Federle, 1Dobbins, Thornton-Manning, and Jones, 1986)

Soil Type/Horizon	Approximate Depth (cm)	Plate Count (CFU/g)
A	0 to 20	10^5 to 10^8
Ap	0 to 12	7.1×10^6
Sterrett/B23t	100 to 125	1.3×10^6
Dewey/B23t	50 to 120	2.5×10^5
Whitwell/C	95 to 200	9.9×10^4
Nauvoo/B3t	80 to 105	4.7×10^4

Other investigations used two methods for measuring bacterial populations in soil at different depths (Novak, Goldsmith, Benoit, and O'Brien, 1985). These detected considerable differences among plate counts but little variation in direct counts with depth. These counts are presented in Table A.3-4 for samples taken from different sites.

Table A.3-4. Bacterial Populations in Subsurface Soils (Novak, Goldsmith, Benoit, and O'Brien, 1985)

Depth (m)	Soil Extract (CFU/g)	AO Direct Count (Organisms/g)
Site 1		
0	9.7×10^6 +- 5.7×10^5	7.6×10^6 +- 3.0×10^6
3	$< 10^3$	5.4×10^6 +- 2.7×10^6
4.5	3.3×10^6 +- 4.0×10^5	2.3×10^6 +- 1.6×10^6
9	5.6×10^5 +- 7.1×10^3	2.9×10^6 +- 2.5×10^6
Site 2		
0	3.0 +- 0.3×10^7	5.6 +- 1.9×10^7
3-4	3.5 +- 2.1×10^3	3.9 +- 1.4×10^7

A number of studies were conducted to determine bacterial counts in uncontaminated soil. These are summarized in Table A.3-5 and described below.

Table A.3-5. Summary of Viable and Direct Counts in Uncontaminated Soils from Several Studies

Viable Counts (CFU/g)	Direct Counts (Organisms/g)
[a] 8×10^3 to 3.4×10^6	[a] 3.4×10^6 to 9.8×10^6
[b] 1.5×10^5 to 8×10^5	[b] 4×10^6 to 9×10^6
	[b] 1.2×10^7 to 1.6×10^7
[c] 5×10^5	[c] 10^6
	[d] 2.1×10^7

a = One study determined the number of bacteria in the B and C horizons of an undefined soil series by use of fluorescent microscopy (Wilson, McNabb, Balkwill, and Ghiorse, 1983). The number of bacteria did not decline with depth and ranged from 3.4 to 9.8 x 10^6 bacteria/g dry wt soil. The viable cell counts were more variable, ranging from 8 x 10^3 to 3.4 x 10^6.

b = Another investigation used fluorescent microscopy on uncontaminated soil (Webster, Hampton, Wilson, Ghiorse, and Leach, 1985). Total counts ranged from 4 x 10^6 to 9 x 10^6 bacteria/g at one site and 1.2 x 10^7 to 1.6 x 10^7 bacteria/g at another. Only a small percentage (<5 percent) of these cells were actively respiring, as measured by their ability to reduce INT. Based on this, the active bacteria ranged from 1.5 x 10^5 to 8 x 10^5 bacteria/g.

c = The microflora of saturated and unsaturated subsurface samples (depths of 4 to 16 ft) were examined (Balkwill and Ghiorse, 1982). Total cells, determined by epifluorescence (EF) light microscopy counts of acridine orange-stained preparations, numbered 10^6/g dry weight in all samples. The population appeared to be entirely bacterial. The predominant cell types were small, coccoid rods, mainly gram positive. Plating on soil extract agar showed that at least 50 percent of the cells counted by EF were viable. Counts on a nutritionally rich medium were three to five orders of magnitude lower.

d = High permeability subsurface soils in a pristine area contained 2.1 x 10^7 cells/g dry soil using acridine orange direct counts (Thomas, Lee, Scott, and Ward, 1986).

3.1.1.5 Counts in Contaminated Soil

Direct counts in contaminated soil were found to range from 10^3 to 10^8 organisms/g, while viable counts were reported from less than 100 to 10^6 CFU/g. These results were derived from the studies below and are summarized in Table A.3-6.

Table A.3-6. Summary of Viable and Direct Counts in Contaminated Soils from Several Studies

Viable Counts (CFU/g)	Direct Counts (Organisms/g)	Hydrocarbon-degraders (Organisms/g)
[a] 10^3 to 10^5	[a] 7.8×10^6	[a] 8.5×10^5
		[a] 1.2×10^5
	[b] 10^6	
[c] <100 to 7×10^6	[c] 7.6×10^6 to 1.7×10^8	
		[d] 10^3

a = High permeability subsurface soils in an area contaminated with jet fuel contained 7.8×10^6 cells/g dry soil using acridine orange direct counts (Thomas, Lee, Scott, and Ward, 1986). Viable counts were one to three orders of magnitude lower; they were higher in contaminated than uncontaminated soil. Additions of 1000 ppb of benzene and 1000, 100, 10, and 1 ppb toluene could not be detected after 4 weeks. The MPN of benzene and toluene degraders in contaminated soil was 8.5×10^5 and 1.2×10^5 cells/g dry soil, while none of these organisms were detected in uncontaminated soil. This indicates the microflora exposed to jet fuel have adapted and multiplied to degrade these compounds.

b = Core samples collected from petroleum-contaminated and uncontaminated soil revealed an even distribution of bacteria for both soil conditions from 0.3 to 2.0 m (10^6 bacteria/gram dry weight of soil) (Stetzenbach, Kelley, Stetzenbach, and Sinclair, 1985). However, bacteria isolated from the contaminated soil were able to degrade naphthalene more quickly in the laboratory than the isolates from the uncontaminated soil. Some PAHs (fluorene, anthracene, pyrene, and naphthalene) were used as sole carbon sources, indicating utilization by the indigenous population.

c = In contaminated soil from Kelly Air Force Base, direct counts of organisms from subsurface samples ranged from 7.6×10^6 to 1.7×10^8 cells/g; viable cells counts ranged from less than 100 to 7×10^6 cells/g (Wetzel, Davidson, Durst, and Sarno, 1986). Similar yields of cells for seven different substrate media indicated the presence of highly adaptive bacteria.

d = A natural flora of gasoline-utilizing organisms were present at levels of 10^3/ml (Jamison, Raymond, and Hudson, 1975) in an area contaminated with over 3,000 barrels of high-octane gasoline. This population was increased a thousand-fold by supplementing the groundwater with air, inorganic nitrogen, and phosphate salts.

3.1.2 Fungi

Naphthalene oxidation predominates in the order Mucorales, which includes species of Cunninghamella, Syncephalastrum, and Mucor (Cerniglia, Hebert, Szaniszlo, and Gibson, 1978). Cunninghamella elegans oxidizes naphthalene and benzo(a)pyrene (Zajic and Gerson, 1977). Other degradative fungi are Neurospora crassa, Claviceps paspale, and Psilocybe strains.

Phanerochaete chrysosporium is a white-rot fungus with a demonstrated ability to degrade chlorinated organics to carbon dioxide in pure liquid culture (Lamar, Larsen, Kirk, and Glaser, 1987). It has also been shown to slowly degrade benzo(a)pyrene in nutrient nitrogen-deficient cultures (Bumpus, Tien, Wright, and Aust, 1985). This ability suggests that the fungus may have potential as an in situ hazardous waste degrader (Lamar, Larsen Kirk, and Glaser, 1987). Degradation of mixtures of complex hydrocarbons by P. chrysosporium often proceeds faster than the rate of degradation of the pure chemicals (Bumpus, Fernando, Mileski, and Aust, 1987). Toxicity of chemicals to this fungus is rare but can be circumvented by using mature mycelia instead of fungal spores.

Soil type has a significant effect on growth and growth habit (Lamar, Larsen, Kirk, and Glaser, 1987). Nitrogen content appears to play a major role in mediating growth of this fungus in the soils. Increasing soil water potential from -1.5 MPa to -0.03 MPa results in greatly increased growth of this organism. It might benefit from soil water potentials above -0.03 MPa. The fungus is mesophilic and has a temperature optimum of about 30°C on 2 percent malt agar (Kirk, Schultz, Connors, Lorenz, and Zeikus, 1978). Growth of the fungus is significantly greater at 30° and 39°C than at 25°C (Lamar, Larsen, Kirk, and Glaser, 1987). Organic matter content of the soil does not affect growth.

The ability of supplemental glucose to increase the rate and extent of biodegradation of DDT suggests a dependency upon the availability of a carbon source that can serve as a growth substrate (Bumpus, Fernando, Mileski, and Aust, 1987). The glucose may simply allow an increase in the overall rate of fungal metabolism or it may provide the substrate for fungal production of hydrogen peroxide, a required cosubstrate for ligninases that are partly responsible for oxidation of many organopollutants. Bulking agents, such as wood chips or corn cobs, can also serve as a carbon source for the fungus. Several Candida spp. are able to metabolize naphthalene, biphenyl, and benzo(a)pyrene (see Table A.3-1).

A strain of soil yeast, Trichosporon cutaneum, which uses phenol in preference to glucose, has been reported (Shoda and Udaka, 1980). This unusual metabolic response could be valuable in transforming xenobiotics. T. cutaneum has a abbroad specificity of enzyme induction and is, actually, as versatile in aromatic catabolism as any pseudomonad (Dagley, 1981).

3.1.3 Photosynthetic Microorganisms

Some cyanobacteria, e.g., an Oscillatoria sp., can oxidize hydrocarbons, such as naphthalene and biphenyl to a limited extent when growing photoautotrophically (Fewson, 1981). Some green, brown, and red algae and diatoms can partially oxidize hydrocarbons, such as naphthalene.

Species of Oscillatoria, Microcoleus, Anabaena, Cocochloris, Nostoc, Chlorella, Dunaliella, Chlamydomonas, Ulva, Cylindretheca, Ampora, and Porphyridium have been found to be capable of oxidizing naphthalene (Cerniglia, Gibson, and Baalen, 1980b).

A few species, such as Rhodopseudomonas palustris (Gottschalk, 1979) or Rhodocyclus purpureus (Pfennig, 1978b), can use thiosulfate or sulfide as an electron donor in addition to organic compounds. Generally, however, the sulfide concentration must be low (Schnitzer, 1982). R. palustris can use benzoate as the sole substrate under aerobic conditions via respiration or anaerobically by photometabolism (Proctor and Scher, 1960). The enzyme system used by this organism to photometabolize aromatic substrates is inducible and substrate specificity (Dutton and Evans, 1969).

An organic compound may be nontoxic in the amounts that exist free in the water or outside the microbial cell in soil, but if the chemical is subject to bioconcentration, species at higher trophic levels may be harmed (Alexander, 1986). Phototrophic organisms can bioaccumulate hydrophobic compounds (Kobayashi and Rittmann, 1982). The organic compounds that can be accumulated by phototrophs as they break down other organic compounds are shown in Table A.3-7. Bioaccumulation of pollutants is also observed in other organisms (Finnerty, Kennedy, Lockwood, Spurlock, and Young, 1973). For example, Acinetobacter sp., yeasts, and filamentous fungi can accumulate hydrocarbons in their cytoplasm.

Table A.3-7. Bioaccumulation of Anthropogenic Compounds by Phototrophs (Kobayashi and Rittmann, 1982)

Microorganism	Compound
Cyanobacteria	
Microcystis aeruginosa	Benzene, toluene, naphthalene, phenanthrene, pyrene
Algae	
Selanastrum capricornatum	Benzene, toluene, naphthalene, phenanthrene, pyrene

A scheme has been devised to separate and treat refractory compounds (Kobayashi and Rittmann, 1982). Degradable compounds are treated directly by the organisms. Refractory compounds are removed by sorption to microorganisms, concentrated, and may be disposed of by other means, such as burial or incineration. Relatively recalcitrant compounds can be degraded if they are first sorbed. This could be accomplished with algae. Other microorganisms may then further degrade intermediate products.

3.1.4 Higher Life Forms and Predation

3.1.5 Cometabolism

Cometabolism is an important process in the breakdown of polycyclic aromatic hydrocarbons (Texas Research Institute, Inc., 1982). It may play a major role in PAH degradation, such as phenanthrene (PHE), in polluted sediments (Shiaris and Cooney, 1981). In sediments, PHE utilizers ranged from $<5 \times 10^2$ to 5.54×10^5 CFU/g dry sediment, which corresponded to <0.1 to 1.8 percent of the total viable count. PHE cooxidizers ranged from 1×10^2 to 1.12×10^6 CFU/g dry sediment, which corresponded to <0.1 to 1.6 percent of the total viable count.

Soil microorganisms capable of cometabolically degrading a given xenobiotic may not be enriched; i.e., there may not be proliferation at the expense of the xenobiotic substrate (Kaufman, 1983). Therefore, unique techniques may be necessary to isolate such organisms. Such techniques could possible utilize, as a primary substrate, a chemical analog that would permit enrichment of microbial populations having the necessary cometabolic requirements to degrade the xenobiotic.

3.1.6 Microbial Interactions

Mutualistic relationships (all members derive some benefit) are based not only upon growth-factor interdependence, but may also encompass removal of a (toxic) product of metabolism produced by one component of the mixed population and used by another (483), combined metabolic attack (Johanides and Hrsak, 1976), or relief of substrate inhibition (Osman, Bull, and Slater, 1976). Some microorganisms thrive on metabolic products or products from lysis of other organisms, as a result of a commensalistic relationship (Harder, 1981). For example, a Nocardia sp. has been identified as the primary cyclohexane utilizer, but growth occurred only in the presence of an unidentified pseudomonad that provided biotin and possibly other growth factors (Sterling, Watkinson, and Higgins, 1977).

3.2 MICROORGANISMS IN GROUNDWATER

Table A.3-8 lists some of the growth requirements for microorganisms present in groundwater (Bitton and Gerba, 1985).

Table A.3-8. Microbial Growth Requirements in the Groundwater Environment (Bitton and Gerba, 1985)

Parameter	Comments
Bacterial Concentrations	Almost 0 to 10^6/ml
Environmental Factors:	
Temperature:	Increases with depth (3^oC/100 m) May preclude growth at great depths
Osmotic pressure:	May have an effect only in saline aquifers
Hydrostatic pressure:	Not likely to exclude microbial growth
pH:	Not likely to exclude microbial growth
Redox potential:	Certain microorganisms may be excluded in very reduced environments
Role of surfaces:	Biodegradation probably occurs on surfaces in the aquifer material
Growth Requirements:	
Carbon sources:	Inorganic C: Carbonates and bicarbonates Organic C: "Humic" substances and wastewater organics, some of which are recalcitrant
Mode of utilization of organics:	At very low concentrations, organics may be utilized as secondary substrates.
Other elements:	N,P,S,Na,Ca,Mg,...may be present in sufficient quantities to allow growth.
Electron acceptors: (Respiration)	O_2: absent in most deep aquifers, but other electron acceptors, such as NO_3^- and SO_4^{2-} may be available.
Sampling for Groundwater Indigenous Microorganisms	Methodological difficulties due mostly to contamination during drilling

3.2.1 Bacteria

3.2.1.1 Aerobes

3.2.1.2 Anaerobes

3.2.1.3 Counts in Uncontaminated Groundwater

Bacterial populations, measured by acridine orange direct microscopy and by plate count, ranged from nondetectable to as high as 10^7 cells or CFU/g of sediment (Fredrickson and Hicks, 1987). Some of the highest counts were from the Tuscaloosa aquifer that attains depths to 400 m below the surface. The greatest diversity and activity of microorganisms and the highest population densities were observed in the sandy water-bearing strata; whereas the dense, dry-clay layer zones had the least microbiological activity.

Using direct counting methods (acridine orange), bacterial populations were not shown to decline appreciably with depths to 800 ft in some wells (Federle, Dobbins, Thornton-Manning, and Jones, 1986). In contrast, indirect plating methods showed declines in counts to zero with depth, indicating that bacteria persist and even grow at relatively deep depths but that they will not grow on plating media (e.g., are probably fastidius and oligotrophic).

Table A.3-9 shows the similarity of counts recovered from different depths of shallow water-table aquifers and vadose zones (Ghiorse and Balkwill, 1983).

Table A.3-9. Numbers of Organisms in the Subsurface Environment (Ghiorse and Balkwill, 1983)

Site	Depth to Water Table (m)	Subsoil[a]	Just Above Water Table[a]	Just Below Water Table[a]
Lula, OK				
Feb. 1981	3.6	6.8	3.4	6.8
June 1981	6.0	9.8	3.7	3.4
Fort Polk, LA	6.0	3.4	1.3	3.0
"	5.0	7.0	1.3	9.8
Conroe, TX	6.0	0.5	0.3	0.6
Long Island, NY	6.0	---	---	36
"	3.0	170	---	---
Pickett, OK	5.0	---	---	5.2

[a]In millions per gram dry material

Other studies also supported the findings that counts are similar at different depths (Novak, Goldsmith, Benoit, and O'Brien, 1985). Table A.3-10 shows the number of bacteria detected at various levels of the saturated zone in samples taken from different sites.

Table A.3-10. Bacterial Populations in Aerobic and Anaerobic Aquifers (Novak, Goldsmith, Benoit, and O'Brien, 1985)

Depth (m)	Plate Counts Soil Extract (CFU/g)	AO Direct Count (bacteria/g)
Site 1		
15 to 17	$5.2 \times 10^6 \pm 9.2 \times 10^5$	$8.0 \times 10^6 \pm 4.7 \times 10^6$
24 to 25	$9.8 \times 10^5 \pm 5.3 \times 10^4$	$5.4 \times 10^6 \pm 4.1 \times 10^6$
31	$1.1 \times 10^5 \pm 2.8 \times 10^4$	$3.5 \times 10^6 \pm 3.3 \times 10^6$
Site 2		
9	$1.4 \times 10^5 \pm 0.8 \times 10^5$	$4.6 \times 10^7 \pm 2.7 \times 10^7$
Site 3		
0	$1.0 \times 10^7 \pm 4.0 \times 10^6$	$1.0 \times 10^8 \pm 4.1 \times 10^7$
2	$9.3 \times 10^5 \pm 1.1 \times 10^5$	$7.6 \times 10^7 \pm 3.8 \times 10^7$
3 to 4	$1.1 \times 10^6 \pm 8.5 \times 10^4$	$8.0 \times 10^7 \pm 7.4 \times 10^7$

The viable and direct counts obtained in groundwater samples by other researchers are described below and summarized in Table A.3-11. As was observed with soil samples and the previous two studies, direct counts showed greater consistency with depth than did plate counts. Overall, direct counts in the saturated zone averaged 10^6 to 10^7 organism/g, ranging from 10^4 to 10^8 organism/g. On the other hand, the viable counts ranged from 10^{-2} to 10^6 CFU/g.

Table A 3.11. Summary of Viable and Direct Counts in Uncontaminated Groundwater from Several Studies

Viable Count (CFU/ml or /g)	Direct Count (Organisms/ml or /g)
[a] 10^3 to 10^5	
[b] 3.4×10^6 (Feb., 5 m)	[b] 6.8×10^6 (Feb., 5 m)
[b] 3.4×10^6 (June, 5 m)	[b] 4.1×10^5 (June, 5 m)
	[c] 10^6 (shallow)
	[d] 10^4 (20 ft)
	[d] 10^5 (450 ft)
[e] 1.4×10^5 (10 m)	[e] 3.4×10^6 (10 m)
[f] 10^{-2} to 10^2 (4.5 to 5.5 m)	[f] 5.5×10^7 to 2.2×10^8 (4.5 to 5.5 m)
[g] 10^4 to 10^6	[g] 10^6 to 10^7
[h] 7×10^3 to 9×10^3	1.21×10^6

a = Sixty percent of the water samples from 621 wells had a plate count of aerobic bacteria of 10^3 to 10^5, 7 percent exceeded a count of 10^6, and 17 percent had a count of zero (McCabe, Symons, Lee, and Robeck, 1970). This showed that groundwater is not sterile.

b = Similar numbers of organisms were found at levels of 1.2, 3.0, and 5.0 meters, (where the water table was at 3.6 meters and the bedrock at 6.0 meters), with a total count ranging from 3.4×10^6 to 6.8×10^6/g dry material at 5 m, and a viable count ranging from 4.1×10^5 to 3.4×10^6/g dry material at 5 m (Wilson, McNabb, Balkwill, and Ghiorse, 1983). The indigenous bacteria recovered could rapidly degrade toluene.

c = In shallow wells, bacterial concentration was shown to be as high as 10^6 bacteria/ml, using epifluorescence microscopy (Ladd, Ventullo, Wallis, and Costerton, 1982).

d = In wells 20-ft and 450-ft deep, 10^4 and 10^5 bacteria/ml have been found, respectively (Bitton and Gerba, 1985).

e = Groundwater (saturated zone, 10 meters deep) had a microscopic count of 3.4 x 10^6 bacteria/g and an agar plate count of 1.4 x 10^5 CFU/g (Benoit, Allen, and Novak, 1984). The unsaturated soil above the groundwater had a microscopic and plate count of 2.9 x 10^6 and 3.5 x 10^3, respectively (see Table A.3-5).

f = Acridine orange direct counts (AODC) were used to determine a population of 5.5 x 10^7 to 2.2 x 10^8 cells/g of soil from a pristine aquifer (Swindoll, Aelion, Dobbins, Jiang, Long, and Pfaender, 1988). The water table occurred at 3.6 m and the bedrock, at 6 m. A fine sand from a depth of 4.5 to 5.5 m was evaluated. Viable bacteria in this soil varied between 0.01 and 1.0 x 10^2 cells/g. The number of bacteria actually found to be degrading the test substrates in this study was a fraction of the total counts but higher than the viable plate count estimates.

g = Subsurface samples from Oklahoma and Texas contained 10^6 to 10^7 cells/g of subsurface material at depths of 2 to 9 m (Webster, Hampton, Wilson, Ghiorse, and Leach, 1985). Between 1 and 10 percent of the cells were metabolically active, a significant number for modifying pollutants.

h = A groundwater microbial community was found to have counts comparable to those in oligotrophic surface waters (Larson and Ventullo, 1983). AODC counts were 1.21 x 10^6/ml; MPN, 10^4 to 10^5/ml; and PC, 7 to 9 x 10^3/ml. The number of bacteria in Canadian groundwater is similar to that found in a pristine groundwater site in Germany and two- to ten-fold lower than those at a polluted site in the same aquifer. The variation in AODC observed from different geographic areas may be due to differences in sampling methods or inherent variation in the biomass in diverse groundwaters; however, too few data are currently available to generalize on this observation.

3.2.1.4 Counts in Contaminated Groundwater

3.3 MICROORGANISMS IN LAKE, ESTUARINE, AND MARINE ENVIRONMENTS

Organisms in eutrophic lakes and ponds are better able to degrade organic compounds than organisms in oligotrophic systems. A greater number of heterotrophic microorganisms can be supported in dense algal communities, which contributes to hydrocarbon degradation.

Exposure of water environments to natural oil seepages and accidental spillages can rapidly alter the composition of the biological communities (Atlas, Schofield, Morelli, and Cameron, 1976). Some of the changes in composition are beneficial in removing the oil, but they may also result in the disappearance of some members of the ecosystem. The effects may be temporary, but the Arctic biological communities recover slowly, if at all.

For example, water collected from Alaskan coastal waters and incubated with Prudhoe crude oil resulted in an increase in the bacterial populations by several orders of magnitude (Atlas, Schofield, Morelli, and Cameron, 1976). The amoeboid protozoa were replaced by flagellated protozoa, coccoid green algae completely disappeared, diatoms increased, while blue-green and green filamentous algae appeared to be unaffected. On the other hand, the organisms present in water associated with a natural oil seepage were very different. There was no vascular plant cover and, in some areas, no bacteria. Fungi (Rhodotorula sp., Candida sp., and a Mucor sp.) were abundant in the bacteria-free regions, with lichen flourishing in older sections. These populations appeared to be adapted to this environment. The fungi were probably tolerant to both the oil and the low pH.

The immediate impact of a spill of fuel oil #2 on a freshwater ecosystem was studied during a seven-day spill period and a seven-day recovery period (Johnson and Romanenko, 1983). An immediate complete collapse of phytoplanktonic an zoophytoplanktonic populations was found. There were dramatic changes in microbiota of sediments, both in functional and microbial diversity. The bacteria were all small rods and cocci; bacterial oil degraders increased 1000 times and saprophytic bacteria increased 100 times. The dissolved oxygen and redox potential decreased. There was no mortality in the fish population.

When contained oil slicks were floated in Prudhoe Bay in Alaska, the bacterial populations directly underlying the slicks increased, especially when the slick was supplemented with an oleophilic fertilizer (octyl phosphate and paraffinized urea) (Atlas, Schofield, Morelli, and Cameron, 1976). The increase was in the oil-degrading psychrophilic Pseudomonas and Staphyloccus epidermidis, a nonhydrocarbon-utilizing mesophile. These organisms were able to extensively degrade the oil and remove the pollutant. The rest of the normal population was unaffected by the oil. Fungi and algae were in concentrations of less than 10/ml and did not increase, even under the fertilized slicks.

Initial toxicity of fuel components can retard biodegradation. When a mixture of 15 hydrocarbons, representative of those in jet fuels, was added to water and sediment containing natural microbial communities collected from estuarine and freshwater sites, C_6 to C_9 compounds (hexane, cyclohexane, n-heptane, methylcyclohexane, toluene, n-octane, ethylcyclohexane, p-xylene, cumene, 1,3,5-trimethylbenzene, n-tetradecane, and 2,3-dimethylnaphthalene) volatilized quickly (Somerville, Butler, Lee, Bourquin, and Spain, 1983). The fuel mixture was initially toxic, causing a 24-hour lag period. However, after

that time, the less volatile indan, naphthalene, and 2-methylnaphthalene were rapidly biodegraded.

Motorboat oils in river water could be degraded using microorganisms in activated sewage sludge as an inoculum (Ludzack and Kinkead, 1956). Reseeding was necessary to maintain the degradation. Rates of degradation increased when elevated temperatures, increased aeration, nutrient supplementation, and oil emulsification were coupled with seeding.

Table A.3-12 lists the most common hydrocarbon-degrading microorganisms isolated from aquatic environments (Bartha and Atlas, 1977).

3.3.1 Counts

Hydrocarbon utilizers are widely distributed, being found in similar concentrations in temperate to Arctic marine environments (Atlas, 1978a). Multiplication of marine bacteria depends upon the composition of the medium and on temperature (Butkevich and Butkevich, 1936). A considerable portion of the bacteria in the sea are probably present in a resting stage.

Approximately 100 oil-degrading bacteria per liter were found in most arctic coastal waters tested (Atlas and Busdosh, 1976). The largest number was 500/l from a saltwater pond, and the smallest was 2/l from a freshwater pond. Populations of hydrocarbonoclastic microorganisms were found to occur in concentrations 10 to 100 times greater in the surface ocean layer than at a 10-cm depth (Crow, Cook, Ahearn, and Bourquin, 1976).

The distribution of hydrocarbon-utilizing microorganisms reflects the historical exposure of the environment to hydrocarbons (Bartha and Atlas, 1977). In polluted sediments, hydrocarbon degraders reached 10^8 CFU/ml (ZoBell and Prokop, 1966). In polluted seawater, up to 10^6 CFU/ml were reported (Polyakova, 1962). In marine environments, hydrocarbon degraders have been found in higher concentrations in regions chronically polluted with petroleum (Environmental Protection Agency, 1985b). Increased rates of uptake and mineralization of [^{14}C]hexadecane were observed for bacteria in samples collected from an oil-polluted harbor compared with samples from a relatively unpolluted, shellfish-harvesting area, with turnover times of 15 and 60 min for these areas, respectively (Walker and Colwell, 1976b). Hydrocarbon-utilizing microbial populations were five to 12 times greater in lake sediment after chronic petrogenic chemical exposure than in sediment from an uncontaminated ecosystem (Heitkamp, Freeman, and Cerniglia, 1987). The fraction of the total heterotrophic bacteria represented by the hydrocarbon utilizers ranged up to 100 percent, depending upon the area's previous history of oil spillage; most values were less than 10 percent (Mulkins-Phillips and Stewart, 1974a). Comparison of data from various investigators is difficult because of the widely varying enumeration techniques employed (Bartha and Atlas, 1977).

Table A.3-12. Hydrocarbon-degrading Microorganisms Isolated from Aquatic Environments (Bartha and Atlas, 1977)

Bacteria	Fungi	Algae
Achromobacter	*Aspergillus*	*Cytophaga*[d]
Acinetobacter	*Aureobasidium*	*Prototheca*
Actinomyces	*Candida*	
Aeromonas	*C. guilliermondii*[c]	
Alcaligenes	*C. lipolytica*[c]	
Arthrobacter	*C. parapsilosis*[c]	
Bacillus	*C. tropicalis*[c]	
Bacterium	*Cephalosporium*	
Beneckea	*Cladosporium*	
Brevibacterium	*Cunninghamella*	
Cladosporium resinae[a]	*Debaryomyces*[c]	
Corynebacterium	*Endomycopsis*[c]	
Flavobacterium	*Hansenula*	
Micrococcus	*Penicillium*	
Mycobacterium	*Pichia*[c]	
Nocardia	*Rhodosporidium*	
Proactinomyces	*R. toruloides*[c]	
Pseudobacterium	*Rhodotorula*	
Pseudomonas	*Saccharomyces*	
Sarcina	*Sporobolomyces*	
Spirillum	*Torulopsis*	
Staphylococcus epidermidis[b]	*Trichosporon*[c]	
Thermomicrobium[e]		
Vibrio		
Xanthomonas[d]		

a = (Colwell, Walker, and Nelson, 1973)
b = (Atlas, Schofield, Morelli, and Cameron, 1976)
c = (Ahearn, Meyers, and Standard, 1971)
d = (Atlas, 1981)
e = (Merkel, Stapleton, and Perry, 1978)

SECTION 4

MICROBIAL DEGRADATION AND TRANSFORMATION OF PETROLEUM CONSTITUENTS AND RELATED ELEMENTS

Microorganisms require energy to maintain themselves (Alexander, 1980). They must carry out oxidations to obtain sufficient energy for their essential functions. Different microorganisms utilize different metabolic processes to derive their energy, and based on what they use for an energy source, these organisms can be classified as autotrophic or heterotrophic (Davis, Dulbecco, Eisen, Ginsberg, and Wood, 1970).

Autotrophic bacteria are able to live in a strictly inorganic environment, utilizing carbon dioxide or carbonates as a sole source of carbon (Zinsser, 1960). The chemosynthetic autotrophs (also called lithotrophs) obtain their energy by oxidation of an inorganic substrate (e.g., iron, sulfur, ammonia, nitrite). Included with the chemoautotrophs are the hydrogen bacteria, which derive energy from the oxidation of hydrogen, and the iron bacteria, which oxidize ferrous salts to ferric hydroxide. Those organisms that can oxidize hydrogen can often similarly utilize carbon compounds (Davis, Dulbecco, Eisen, Ginsberg, and Wood, 1970).

The photosynthetic autotrophs are pigmented anaerobic organisms that obtain energy by the utilization of radiant energy (Zinsser, 1960). There are three kinds of photosynthetic bacteria: purple suflur bacteria, which reduce CO_2 at the expense of H_2S; purple nonsulfur bacteria, which reduce CO_2 at the expense of organic compounds, require growth factors, and cannot use H_2S as a hydrogen donor; and green sulfur bacteria, which reduce CO_2 at the expense of H_2S. Bacterial photosynthesis differs from the process in green plants in that molecular oxygen is not produced. Also, in green plants, the hydrogen donor is H_2O, while in bacteria, a variety of oxidizable substances may be utilized.

Heterotrophic aerobes employ respiration to oxidize organic compounds as a source of carbon and energy. The heterotrophic bacteria require a more complex source of carbon than CO_2 and obtain their energy by the degradation of organic matter. These organisms are the most active in degradation of petroleum hydrocarbons.

Table A.4-1 presents examples of autotrophic modes of metabolism.

Table A.4-2 summarizes several microbial processes and shows an approximate relationship between the process and the environmental redox potential (Berry, Francis, and Bollag, 1987). The more negative the number, the stronger the reducing environment, as can be seen by the strict requirement for anaerobiosis.

4.1 ORGANIC COMPOUNDS

Table A.4-3 lists the aromatic hydrocarbons known to be oxidizable by microorganisms (Gibson, 1977). Individual components of petroleum and organisms capable of degrading them are given in Table A.4-4, originally

Table A.4-1. Autotrophic Modes of Metabolism (Davis, Dulbecco, Eisen, Ginsberg, and Wood, 1970)

Organism or Group	Source of Energy	Remarks
Aerobic lithotrophs		Inorganic (litho-) electron donors
Hydrogen bacteria	$H_2 + 1/2\ O_2 \rightarrow H_2O$	
Sulfur bacteria (colorless)	$H_2S + 1.2\ O_2 \rightarrow H_2O + S$ $S + 1.5\ O_2 + H_2O \rightarrow H_2SO_4$	Can produce H_2SO_4 to pH as low as 0
Iron bacteria	$2\ Fe^{+2} + 1/2\ O_2 + H_2O \rightarrow 2\ Fe^{+3} + 2\ OH^-$	
Nitrifying bacteria		
Nitrosomonas	$NH_3 + 1.5\ O_2 \rightarrow HNO_2 + H_2O$	Convert soil N to nonvolatile form Most can also use electron donors
Nitrobacter	$HNO_2 + 1/2\ O_2 \rightarrow HNO_3$	
Anaerobic respirers		
Denitrifiers[a]	$nH_2 + NO_3^- \rightarrow N_2O$, N_2, or NH_3	Cause N loss from anaerobic soil
Desulfovibrio	$nH_2 + SO_4^{-2} \rightarrow S$ or H_2S	Odor of polluted streams, mud flat
Methane forming bacteria	$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$	Sewage disposal plants
Clostridium aceticum	$4\ H_2 + 2\ CO_2 \rightarrow CH_3COOH + 2\ H_2O$	
Photosynthesizers		
Purple sulfur bacteria	$4\ CO_2 + 2\ H_2S + 4\ H_2O \xrightarrow{light} 4\ (CH_2O) + 2\ H_2SO_4$	$H_2(A)$ = various electron donors
Nonsulfur purple	$CO_2 + 2\ H_2(A) \xrightarrow{light} (CH_2O) + H_2O + 2\ (A)$	
Algae	$CO_2 + 2\ H_2O \xrightarrow{light} (CH_2O) + 1/2\ O_2$[b]	Plant photosynthesis

a = Anaerobic respiration, with the use of nitrate instead of O_2, is also common for the oxidation of the usual organic substrates by heterotrophs. This metabolism bears no resemblance to autotrophy, as the energy is used for biosynthesis from organic compounds rather than from CO_2.

b = The O_2 is derived directly from H_2O, and not from CO_2.

Table A.4-2. Relationship Between Representative Microbial Processes and Redox Potential (Berry, Francis, and Bollag, 1987)

Process	Reaction (electron donor + electron acceptor)	Physiological Type	Redox Potential (mV)
Respiration	OM + O_2 --- CO_2	Aerobes	700 to 500
Denitrification	OM + NO_3^- --- N_2 + CO_2	Facultative anaerobes	300
Fermentation	OM --- organic acids (mostly acetate, propionate, and butyrate)	Facultative or obligate anaerobes	
Dissimilatory sulfate reduction	OM (or H_2) + SO_4^{-2} --- H_2S + CO_2	Obligate anaerobes	-200
Proton reduction	OM (C_4 to C_8 FA) + H_2 + acetate (propionate) + CO_2	Obligate anaerobes	
Methanogenesis	CO_2 + H_2 --- CH_4 Acetate --- CO_2 + CH_4	Obligate anaerobes	<-200 <-200

OM = Organic matter
FA = Fatty acid

Table A.4-3. Aromatic Hydrocarbons Known to be Oxidized by Microorganisms (Gibson, 1977)

Monocyclic	Tricyclic
Benzene	Phenanthrene
Toluene	Anthracene
Xylenes	
Tri- and Tetramethylbenzenes	
Alkylbenzenes (Linear and Branched)	Polycyclic
Cycloalkylbenzenes	Pyrene
	Benzo(a)pyrene
	Benzo(a)anthracene
Dicyclic	Dibenzo(a)anthracene
Naphthalene	Benzperylene
Methylnaphthalenes (Mono and Di)	

Table A.4-4. Fuel Components/Hydrocarbons and Microorganisms Capable of Biodegrading/Biotransforming Them

Fuel Component/ Hydrocarbon	Microorganisms
Acrylonitrile	*Mixed culture of yeast, mold, protozoa, bacteria; activated sludge*[e]
Alkanes	*Pseudomonas*[p], *Arthrobacter, Acinetobacter,* yeasts, *Penicillium* sp., *Cunninghamella blakesleeana, Absidia glauca, Mucor* sp.[f]
n-Alkanes (C_1 to C_4) gaseous	*Mycobacterium ketoglutamicum*[f]
n-Alkanes (C_3 to C_{16})	*Mycobacterium rhodochrous*[g]
n-Alkanes (C_8 to C_{16})	*Mycobacterium fortuitum, M. smegmatis*[g]
n-Alkanes (C_{12} to C_{16})	*Mycobacterium marinum, M. tuberculosis*[g]
n-Alkanes (C_5 to C_{16})	*Corynebacterium*[f] *(Arthrobacter, Acinetobacter, Pseudomonas putida,* yeasts)[f]
n-*Alkanes (C_{10} to C_{14})*	*Corynebacterium*[g]
n-Alkanes (C_8 to C_{20})	*Acinetobacter*[q]
n-Alkanes (C_{11} to C_{19})	*Prototheca zopfii*[l], *Pseudomonas* spp[k,m]
Alkanes (straight chain)	*Pseudomonas putida*[f]
Alkenes (C_6 to C_{12})	*Pseudomonas oleovorans*[ab]
Anthracene	Stream bacteria[e], *(Flavobacterium, Beijerinckia* sp., *Cunninghamella elegans)*[h] *(Pseudomonas/Alcaligenes* sp, *Acinetobacter* sp., *Arthrobacter* sp.)[k]
Aromatics	*Pseudomonas* sp.[j]
Benzene	*Pseudomonas putida*[o,h,ae], sewage sludge[e], stabilization pond microbes[e], *P. rhodochrous*[f], *P. aeruginosa*[f] methanogens[r,s], anaerobes[t], *Acinetobacter* sp.[ae], *Methylosinus trichosporium* OB3b[ag], *Nocardia* sp.[ah],
Benzo(a)anthracene	*Beijerinckia* sp[f].[c,g], *Cunninghamella elegans*[e,u] *Pseudomonas* sp.[f]
Benzo(a)pyrene	*(Candida lipolytica, C. tropicalis, C. guilliermondii, C. maltosa, Debaryomyces hansenii)*[a], *Bacillus megaterium*[b] *Beijerinckia* sp.[c,g], *Cunninghamella elegans*[a,u,ap], *Pseudomonas* sp.[a], *Neurospora crassa*[ai],

Table A.4-4. Fuel Components/Hydrocarbons and Microorganisms Capable of Biodegrading/Biotransforming Them (Continued)

Fuel Component/ Hydrocarbon	Microorganisms
	Saccharomyces cerevisiae[aj]
Biphenyl	*(Candida lipolytica, C. tropicalis, C. Guilliermondii, C. maltosa, Debaryomyces hansenii)*[a], *(Beijerinckia* B8/36, *Oscillatoria* sp., *Pseudomonas putida*[e,ae], *Cunninghamella elegans*[h], *(Moraxella* sp., *Pseudomonas* sp., *Flavobacterium* sp.)[ad], *Beijerinckia* sp.[ae], *Oscillatoria* sp.[ap]
n-Butane	*Mycobacterium smegmatis, Pseudobacterium subluteum, Pseudomonas fluorescens, Actinomyces candidus*[g], *(Arthrobacter, Brevibacterium*[f]
Chlorobenzene	*Pseudomonas putida*[ae]
Cresols	*Methylosinum trichosporium* OB3b[ag]
p-Cresol	*Pseudomonas* sp.[al]
Cyclohexane	*Xanthobacter* sp., *Nocardia* sp.[h]
Cyclohexanol	*Xanthobacter autotrophicus*[ak], *(Acinetobacter, Nocardia globerula)*[h]
Cyclohexanone	*Xanthobacter autotrophicus*[ak]
Decane	*Corynebacterium*[f]
Dibenzanthracene	Activated sludge[a]
Dodecane	*(Arthrobacter, Acinetobacter, Pseudomonas putida*, yeasts[f]
Ethane	*Methylosinus trichosporium*[f], *Pseudomonas methanica*[g], *P. putida*[h]
Ethylbenzene	*Pseudomonas putida*[ae,o]
Fluoranthene	Sewage sludge[e] *Pseudomonas* spp[m]
n-Heptane	*Pseudomonas aeruginosa*[g], *(Arthrobacter, Acinetobacter, Pseudomonas putida*, yeasts)[f]

Table A.4-4. Fuel Components/Hydrocarbons and Microorganisms Capable of Biodegrading/Biotransforming Them (Continued)

Fuel Component/ Hydrocarbon	Microorganisms
n-Hexane	*Mycobacterium smegmatis*[g]
Hexadecane	*Acinetobacter* sp.[f], *(Candida petrophilum, Pseudomonas aeruginosa, Arthrobacter* sp.)[f], *Micrococcus cerificans (Candida parapsilosis, C. tropicalis, C. guilliermondii, C. lipolytica, Trichosporon* sp., *Rhodosporidium toruloides)*[l], *Prototheca zopfii (alga)*[l], *(Pseudomonas putida,* yeasts)[f], *Nocardia* sp.[ac], *(Pichia, Debaryomyces, Torulopsis, Candida*[an]
Jet fuels	*Cladosporium, Hormodendrum*
Kerosene	*Torulopsis, Candida tropicalis, Corynebacterium hydrocarboclastus (Candida parapsilosis, C. guilliermondii, C. lipolytica, Trichosporon* sp., *Rhohosporidium toruloides)*[l], *Cladosporium resinae*[ao]
Kerosene, Jet fuel, Paraffin wax	*Aspergillus, Botrytis, Candida, Cladosporium, Debaromyces, Endomyces, Fusarium, Hansenula, Monilia, Penicillium, Actinomyces, Micromonospora, Nocardia, Proactinomyces, Streptomyces,*
Methane	*Pseudomonas methanica*[g]
2-Methylhexane	*Pseudomonas aeruginosa*[g]
Octadecane	*Micrococcus cerificans*[g]
Naphthalene	*Pseudomonas* sp.[p], *(Candida lipolytica, C. tropicalis, C. Guilliermondii, C. maltosa, Debaryomyces hansenii)*[a], *Cunninghamella bainieri*[c,h], *Cunninghamella elegans*[c,e,h] *(Agnenellum, Oscillatoria, Anabaena, Cunninghamella elegans, Microcoleus* sp., *Nostoc* sp., *Coccochloris* sp., *Aphanocapsa* sp., *Chlorella* sp., *Dunaliella* sp., *Chlamydamonas* sp., *Cylindriotheca* sp., *Amphora* sp., *Flavobacterium, Alcaligenes, Corynebacterium, Nocardia,*

Table A.4-4. Fuel Components/Hydrocarbons and Microorganisms Capable of Biodegrading/Biotransforming Them (Continued)

Fuel Component/ Hydrocarbon	Microorganisms
	Aeromonas, stream bacteria)[e] *Pseudomonas rathonis*[g], *(Bacillus naphthalinicum nonliquifaciciens, Pseudomonas desmolyticum, P. fluorescens, P. putida* biotype B)[h], *Pseudomonas oleovorans*[g], *P. putida*[f,v], (Mucorales: *Cunninghamella elegans, C. echinulata, C. japonica, Syncephalastrum* sp., *S. racemosum, Mucor* sp., *M. hiemalis, Neurospora crassa, Claviceps paspali, Psilocybe strictipes, P. subaeruginascens, P. cubensis, P. stuntzii)*[aa] *(Pseudomonas* NCIB 9816, *P.* sp. 53/1 and 53/2, *P. desmolyticum, Nocardia* strain R, *Nocardia* sp. NRRL 3385)[ae] Cyanobacteria[ap]
Octane	*Pseudomonas putida*[f,v], *Corynebacterium* sp. 7EIC[f], *Pseudomonas*[g]
Paraffins	*Trichosporon pullulans Nocardia* sp.[j]
n-Pentane	*Mycobacterium smegmatis*[g]
Phenanthrene	*Beijerinckia*[e], *(Pseudomonas putida, Cunninghamella elegans)*[h], *Pseudomonas* spp.[m], *Flavobacterium*[h,w]
Phenol	*(Pseudomonas, Vibrio, Spirillum, Bacillus, Flavobacterium, Chromobacter, Nocardia, Chlamydamonas ulvaensis, Phoridium fuveolarum, Scenedesmus basiliensis, Euglena gracilus, Corynebacterium* sp.)[e] *(Pseudomonas putida*, yeasts)[m], *(Azotobacter* sp., *Pseudomonas putida* CB-173 (ATCC 31800)[ab] *Acinetobacter calcoaceticus*[af]
Pristane	*(Corynebacterium* sp, *Brevibacterium erythrogenes*[f]
n-Propane	*Mycobacterium smegmatis, M. rubrum, M. rubrum var. propanicum, M. carotenum, Pseudomonas puntotropha, (Pseudobacterium subluteum, Pseudomonas methanica)*[g],

Table A.4-4. Fuel Components/Hydrocarbons and Microorganisms Capable of Biodegrading/Biotransforming Them (Continued)

Fuel Component/ Hydrocarbon	Microorganisms
	(Cunninghamella elegans, Penicillium onatum)[f]
1-Propanol >2-propanol	*(Nocardia paraffinica, Brevibacterium* sp)[f]
Pyrene	Stabilization pond organisms[e] *(Pseudomonas/Alcaligenes* sp., *Acinetobacter* sp., *Arthrobacter* sp)[k]
Tetradecane	*Micrococcus cerificans*[g] *(Arthrobacter, Acinetobacter, Pseudomonas putida*, yeasts[f]
Toluene	*Bacillus* sp.[e], *Pseudomonas putida*[e,f,o,m,ae], *Cunninghamella elegans*[n], *(P. aeruginosa, P. mildenberger)*[f] methanogens[r,s], anaerobes[s,y,t], *Methylosinus trichosporium* OB3b[ag], *(Pseudomonas* sp., *Achromobacter* sp.)[ah], *Pseudomonas aeruginosa*[am]
n-Undecane	*Mycobacterium* sp.[g]
p- and *m*-Xylene	*Pseudomonas putida*[f,ae], methanogens[n], anaerobes[y,t]

References:

a = (Cerniglia and Crow, 1981)
b = (Pogıazova, Fedoseeva, Khesina, Meissel, and Shabad, 1967)
c = (Gibson, Mahadevan, Jerina, Yagi, and Yeh, 1975)
d = (Magor, Warburton, Trower, and Griffin, 1986)
e = (Kobayashi and Rittmann, 1982)
f = (Hou, 1982)
g = (Zajic, 1964)
h = (Cerniglia and Gibson, 1977)
i = (Ahearn, Meyers, and Standard, 1971)
j = (Jamison, Raymond, and Hudson, 1975)
k = (Stetzenbach and Sinclair, 1986)
l = (Boehn and Pore, 1984)
m = (Ghisalba, 1983)
n = (Reinhard, Goodman, and Barker, 1984)
o = (Gibson, Koch, and Kallio, 1968)

Table A.4-4. References (Continued)

p = (Solanas, Pares, Bayona, and Albaiges, 1984)
q = (Garvey, Stewart, and Yall, 1985)
r = (Grbic-Galic and Vogel, 1986)
s = (Grbic-Galic and Vogel, 1987)
t = (Battermann and Werner, 1984)
u = (Dodge and Gibson, 1980)
v = (Jain and Sayler, 1987)
w = (Foght and Westlake, 1985)
x = (Rees, Wilson, and Wilson, 1985)
y = (Zeyer, Kuhn, and Schwarzenback, 1986)
aa = (Cerniglia, Hebert, Szaniszlo, and Gibson, 1978)
ab = (Roberts, Koff, and Karr, 1988)
ac = (Mulkins-Phillips and Stewart, 1974b)
ad = (Stucki and Alexander, 1987)
ae = (Knox, Canter, Kincannon, Stover, and Ward, 1968)
af = (Fewson, 1981)
ag = (Higgins, Best, and Hammond, 1980)
ah = (Claus and Walker, 1964)
ai = (Lin and Kapoor, 1979)
aj = (Wiseman, Lim, and Woods, 1978)
ak = (Magor, Warburton, Trower, and Griffin, 1986)
al = (Dagley and Patel, 1957)
am = (Kitagawa, 1956)
an = (Scheda and Bos, 1966)
ao = (Atlas, 1977)
ap = (Atlas, 1981)

published by Poglazova, Fedoseeva, Khesina, Meissel, and Shabad, 1967, and expanded in this report by inclusion of additional references. Thirty-two organisms were isolated from groundwater contaminated with high-octane gasoline (Jamison, Raymond, and Hudson, 1976). These were identified and used to study biodegradation of selected constituents of the gasoline (Table A.4-5). Table A.4-6 shows the biodegradation of gasoline components by mixed normal flora (Jamison, Raymond, and Hudson, 1976). The mixed population of natural flora in the groundwater biodegraded more constituents of the gasoline than the individual isolates. This suggests that a form of mutualism takes place in the degradation of petroleum, where a variety of organisms are necessary for complete degradation. The percent of each constituent biodegraded by the mixture is given in the table.

4.1.1 Aerobic Degradation

Microorganisms have evolved catabolic enzyme systems for metabolism of naturally occurring aromatic compounds (Gibson, 1978). In the oxidation of aromatic hydrocarbons, oxygen is the key to the hydroxylation and fission of the aromatic ring. Two hydroxyl groups must be present on the aromatic nucleus for enzymatic fission of the ring to occur, and these may be ortho (adjacent) or para (opposite on the ring) to each other. Subsequent metabolic sequences will then vary, depending upon the organism and the site of ring cleavage. Man-made molecules are degraded by these enzymes, if they are structurally similar to the naturally occurring compounds.

Bacteria incorporate two atoms of oxygen into the hydrocarbons to form dihydrodiol intermediates (Gibson, 1978). The hydroxyl groups are cis-dihydrodiols; i.e., they have a cis-stereochemistry. Oxidation of the dihydrodiols leads to the formation of catechols, which are substrates for enzymatic cleavage of the aromatic ring. In contrast, certain strains of fungi and higher organisms (Eukaryotes) incorporate one atom of molecular oxygen into aromatic hydrocarbons to form arene oxides, which can undergo the enzymatic addition of water to yield trans-dihydrodiols. These differences are illustrated in Figure A.4-1.

For most compounds, the most rapid and complete degradation occurs aerobically (Environmental Protection Agency, 1985b). It can be generalized that for the degradation of petroleum hydrocarbons, aromatics, halogenated aromatics, polyaromatic hydrocarbons, phenols, halophenols, biphenyls, organophosphates, and most pesticides and herbicides, aerobic bioreclamation techniques are most suitable. Aerobic degradation with methane gas as the primary substrate appears promising for some low molecular-weight halogenated hydrocarbons.

The sequence of hydrocarbon degradation in an oil spill is also likely to be determined by the ecological succession of the degrading microorganisms (Bartha and Atlas, 1977). n-Alkane degraders with rapid growth rates would out-compete the slow-growing decomposers of the more recalcitrant hydrocarbons for the nutritional resources until the n-alkanes are depleted. These organisms would then be replaced by microbes with slower growth rates but greater metabolic flexibility to degrade the more recalcitrant hydrocarbons (Fredericks, 1966).

Table A.4-5. Growth of Microorganisms on Components of Gasoline (Jamison, Raymond, and Hudson, 1976)

Compound	Nocardia	Pseudo-monas	Acineto-bacter	Micro-coccus	Flavo-bacterium	Unclas-sified
General Compound Classes						
n-Alkanes	+	-	-	+	-	-
Cyclic alkanes	-	-	-	-	-	-
Alkyl-substituted cyclicalkane	-	-	-	-	-	-
Monomethylalkanes	+	-	-	+	+	-
Dimethylalkanes	-	-	-	-	+	-
Trimethylalkanes	+	+	+	-	-	+
Aromatics	-	+	+	+	-	+
Specific Compounds						
n-Butane	-	-	-	-	-	-
n-Pentane	*+-	-+*	-	-+	-	-+
n-Hexane	*+	-+*	-+	-+	-	-
n-Heptane	*+-	-+*	-	-	-	-+
n-Octane	*+-	-+*	-	-	-	-+*
n-*cis*-Butene-2	-	-	-	-	-	-
n-Pentane-2	-	-	-	-	-	-
2,3-Dimethyl-butene-1	-	-	-	-	-	-
Cyclopentane	-	-	-	-	-	-
Cyclohexane	-	-	-	-	-	-
Methylcyclo-pentane	-	-	-	-	-	-
2-Methylbutane	-+*	-+*	-	-	-	-
2-Methylpentane	-+*	-	-+	+	-	-+
3-Methylpentane	-	-	-	-	-	-
3-Methylhexane	-	-	-	-	-	-
2-Methylheptane	-	-	-	-	-	-
3-Methylheptane	*+-	-+	-	-	-	-
2,2-Dimethyl-butane	-	-	-	-	-	-
2,3-Dimethyl-butane	-	-	-	-	-	-
2,2-Dimethyl-pentane	-	-	-	-	-	-
2,4-Dimethyl-pentane	-	-	-	-	-	-
2,3-Dimethyl-pentane	-	-	-	-	-	-
2,3-Dimethyl-hexane	-	-	-+	-+	+	-+
1,2-Dimethyl-cyclohexane	-	-	-	-	-	-

Table A.4-5. Growth of Microorganisms on Components of Gasoline (Jamison, Raymond, and Hudson, 1976) (Continued)

Compound	Nocardia	Pseudo-monas	Acineto-bacter	Micro-coccus	Flavo-bacterium	Unclas-sified
2,2,4-Tri-methylpentane (isooctane)	*+-	-+	*+-	-	-	*+-
2,3,4-Tri-methylpentane (isooctane)	-	-	-	-	-	-
2,3,3-Tri-methylpentane (isooctane)	-	-	-	-	-	-
Ethylcyclohexane	-	-	-	-	-	-
Benzene	-+*	-	+*	-	-	-+*
Ethylbenzene	+-*	-	+*	-+	-	-+
Toluene	-+	+*	+	+-	-	-+*
o-Xylene	-	-	-	-	-	-
m-Xylene	-	+-	-	-	-	-
p-Xylene	-	+-	-	-	-	-
Gasoline	+*	+*	+*	+	+-	+*

Growth on the Specific Compounds:
+- most isolates were +
-+ most isolates were -
* some isolates exhibited moderate to heavy growth

Table A.4-6. Biodegradation of Gasoline Components by Mixed Normal Microflora (Jamison, Raymond, and Hudson, 1976)

Component	Percent Biodegraded above Control	Component	Percent Biodegraded above Control
n-Propane	0	2,3-Dimethylpentane	0
n-Butane	0	2,5-Dimethylhexane	20
n-Pentane	70	2,4-Dimethylhexane	0
n-Hexane	46	2,3-Dimethylhexane	19
n-Heptane	49	3,4-Dimethylhexane	84
n-Octane	54	2,2-Dimethylhexane	75
Olefins--C_4	0	2,2-Dimethylheptane	62
Olefins--C_5	16	1,1-Dimethylcyclopentane	25
Olefins--C_6	18	1,2- and 1,3-Dimethylcyclopentane	78
Isobutane	0	1,3- and 1,4-Dimethylcyclohexane	0
Cyclopentane	0	1,2-Dimethylcyclohexane	26
Cyclohexane	45	2,2,3-Trimethylbutane	62
Methylcyclopentane	10	2,2,4-Trimethylpentane	13
Methylcyclohexane	75	2,2,3-Trimethylpentane	54
2-Methylbutane	0	2,3,4-Trimethylpentane	13
2-Methylpentane	6	2,3,3-Trimethylpentane	16
3-Methylpentane	7	2,2,5-Trimethylpentane	23
2-Methylhexane	23	1,2,4-Trimethylcyclopentane	0
3-Methylhexane	0	Ethylpentane	0
2-Methylheptane	38	Ethylcyclopentane	31
3-Methylheptane	45	Ethylcyclohexane	95
4-Methylheptane	48	Benzene	100
2,2-Dimethylbutane	25	Ethylbenzene	100
2,3-Dimethylbutane	0	Toluene	100
2,2-Dimethylpentane	9	o-Xylene	100
2,4-Dimethylpentane	11	m-Xylene	100
3,3-Dimethylpentane	45	p-Xylene	100
		Heavy ends	87

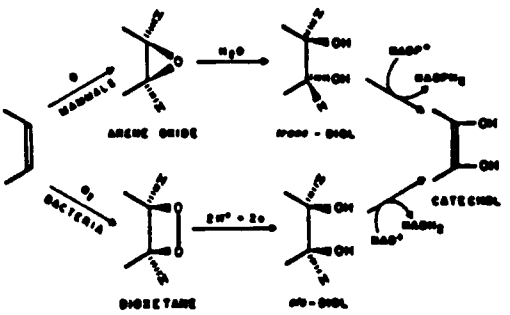


Figure A.4-1. Differences Between the Reactions Used by Eukaryotic and Procaryotic Organisms to Initiate the Oxidation of Aromatic Hydrocarbons (Gibson, 1977)

The diversity of catabolic pathways for the degradation of hydrocarbons among different species and even strains of microorganisms makes it difficult to summarize all of the varied mechanisms and reactions that can occur (Hornick, Fisher, and Paolini, 1983). However, some of the major degradative pathways can be discussed.

4.1.1.1 Degradation of Straight Chain Alkanes

The n-Alkanes are the most widely and readily utilized hydrocarbons, with those between C_{10} and C_{25} being most suitable as substrates for microorganisms (Bartha and Atlas, 1977). The process is similar to the degradation of fatty acids.

The straight chain alkanes are oxidized by Pseudomonas spp. after 20 days at 20°C, being converted into the isoprenoids, pristane and phytane, the polycyclic sterane, and 17 alpha (H), 21 beta (H)-hopane series, all of which are resistant to biodegradation (Gibson, Koch, and Kallio, 1968). The n-alkanes with shorter chains (from C_5 to C_9) are more easily used as a source of carbon and energy by microorganisms than those with longer chain lengths (from C_{10} to C_{14}) (Williams, Cumins, Gardener, Palmier, and Rubidge, 1981). Biodegradation of n-alkanes with molecular weights up to n-C_{44} has been demonstrated (Haines and Alexander, 1974).

Oxidation of C_{10} to C_{14} alkanes is obtained with Corynebacterium (Zajic, 1964). Alkanes are metabolized by terminal oxidation, alpha-oxidation, and diterminal oxidation. Fatty acids formed as by-products may be metabolized further by beta-oxidation. C_{12}-C_{16} are oxidized by Corynebacterium and further converted to the corresponding ketones or to esters of lower aliphatic acids (Hou, 1982z). Alkanes (C_5 to C_{16}) have been utilized by selected strains of Arthrobacter, Acinetobacter, Pseudomonas putida, and yeasts. Heptane, dodecane, tridecane, tetradecane, and hexadecane have been reported as growth substrates.

n-Alkane biodegradation is a predominantly bacterial activity (Song and Bartha, 1986). See Section 4.1.3 for the products formed from the microbial oxidation of alkanes.

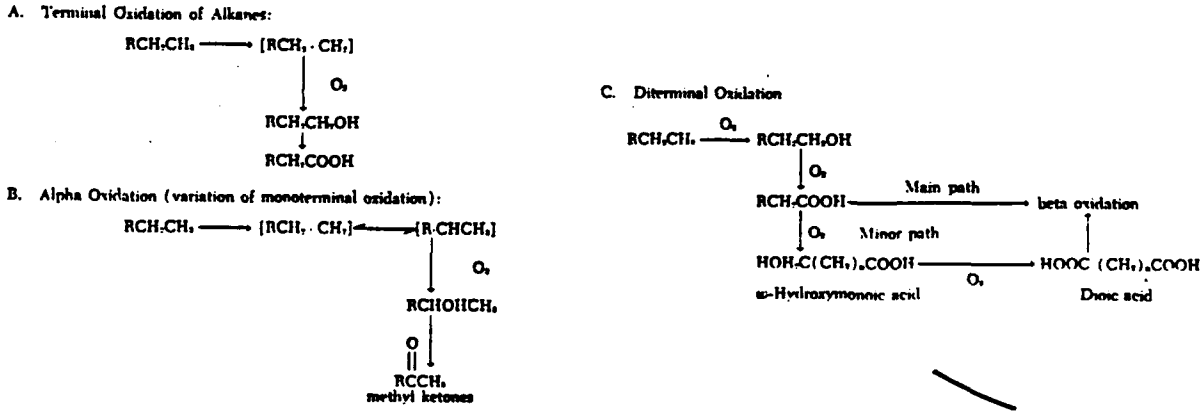
1. Methane

The ability to oxidize small amounts of methane is widely spread among soil microbes. It is oxidized by Pseudomonas methanica (Zajic, 1964). Methane is also oxidized by Mycobacterium fortuitum and M. smegmatis (Lukins, 1962).

2. Ethane, propane, butane

These can be cooxidized by Pseudomonas methanica, with oxygen as a limiting factor (Zajic, 1964). Butane is utilized for growth by Pseudobacterium subluteum, Pseudomonas fluorescens, and Actinomyces candidus (Telegina, 1963).

Oxidation of alkanes or aliphatic hydrocarbons is classified as being terminal or diterminal (Zajic, 1964). These terms indicate that the initial breach occurs at one of the terminal carbon atoms:



Monoterminal oxidation proceeds by the formation of a free radical and then the corresponding alcohol, which is readily oxidized to its respective aldehyde or aliphatic acid (Zajic, 1964). The terminal methyl group is enzymatically oxidized by incorporation of molecular oxygen by a monooxygenase, producing a primary alcohol, with further oxidation to a monocarboxylic acid (Atlas, 1981; Hornick, Fisher, and Paolini, 1983). Beta-oxidation of the carboxylic acid results in formation of fatty acids and acetyl coenzyme A, with eventual liberation of carbon dioxide. Some fatty acids are toxic and have been found to accumulate during hydrocarbon biodegradation. Fatty acids produced from certain chain length alkanes may be directly incorporated into

membrane lipids, instead of going through the beta-oxidation pathway (Dunlap and Perry, 1967).

Alpha-oxidation of alkanes to methyl ketones may proceed with the same initial breech, but at the alpha-carbon (Zajic, 1964). Alpha-alcohols are formed, which are futher oxidized to methyl ketones. Alpha-oxidation has been observed with propane, butane, pentane, and hexane.

Diterminal (omega) oxidation (Kester and Foster, 1963) of C_{10} to C_{14} alkanes has been obtained with a culture of Corynebacterium. A breach occurs first at one terminal carbon atom and is followed by a second breach at the omega-carbon atom, synthesizing first a primary alcohol and then an aliphatic acid. The second breach at the omega carbon gives an omega-hydroxymonoic acid, which is oxidized to the corresponding dioic acid. Certain species of Pseudomonas oxidize the fatty acids formed from heptane and hexane by classical beta-oxidation (Heringa, Huybregtse, and van der linden, 1961). Decarboxylation of fatty acids does not occur in these particular systems.

Subterminal oxidation occurs with C_3 to C_6, and longer, alkanes with formation of a secondary alcohol and subsequent ketone; these may or may not be metabolized. However, this is probably not the primary metabolic pathway for most n-alkane-utilizing microorganisms.

At chain lengths greater than C_6, the degradability generally increases until about C_{11} to C_{12} (Hornick, Fisher, and Paolini, 1983). As the alkane chain length increases, the molecule becomes less soluble in water. However, at chain lengths of C_{11} to C_{12} and above, the liquid n-alkanes are "accommodated" in water at a higher concentration than would be extrapolated from the solubility of a series of lower alkanes. This may be due to a change in the structure of the water molecules surrounding the alkanes. During the microbial utilization of long chain liquid alkanes, microbial attachment to droplets of alkane is seen with "transport" of these long chain alkanes through the cell membrane. This is also thought to occur with solid long chain alkanes, although, in both cases, the actual mechanism is unknown. Entirely separate transport mechanisms may exist for gaseous alkanes, short chain liquid alkanes, "accommodated" intermediate chain liquid alkanes, and long chain solid alkanes (Perry, 1968).

4.1.1.2 Degradation of Branched and Cyclic Alkanes

Only a few microorganisms utilize these hydrocarbons, possibly because the oxidation enzymes are not capable of handling branched-chain substrates (Hou, 1982). The fatty acids resulting from oxidation of single-branched alkanes are incorporated into the cell lipid. Subsequent oxidation is usally then by the beta-oxidation pathway.

Multiple-branched alkanes, such as pristane, can be converted to succinyl-CoA by Corynebacterium sp. and Brevibacterium erythrogenes.

Isoalkanes (branched alkanes) are degradable by a large number of microorganisms, although they are generally inferior to n-alkanes as growth substrates, especially if the branching is extensive or creates quaternary carbon atoms (Hornick, Fisher, and Paolini, 1983). A small number of methyl or ethyl side chains does not drastically decrease the degradability, but complex

branched chains, and especially, terminal branching, are harder to degrade. The position of the side chain also has an effect on the degradability. 1-Phenylalkanes are more degradable than interior substituted ones, and the further the side group is into the molecule, the slower the degradation.

Cycloalkanes are more resistant to microbial attack than straight chain alkanes and are highly toxic (Atlas, 1981). This is probably due to the absence of an exposed terminal methyl group for the initial oxidation (Hornick, Fisher, and Paolini, 1983). Complex alicyclic compounds, such as hopanes, are among the most persistent components of petroleum spillages in the environment. Up to six-membered condensed ring structures have been reported to be subject to microbial degradation. Bacterial degradation of aromatic hydrocarbons normally involves the formation of a diol followed by cleavage and formation of a cis diacid, while that of fungi forms a trans-diol. Light aromatic hydrocarbons are subject to microbial degradation in a dissolved state. Condensed ring aromatic structures are subject to microbial degradation by a similar metabolic pathway as monocyclic structures; however, these hydrocarbons are relatively resistant to enzymic attack. Structures with four or more condensed rings have been shown to be attacked by cooxidation or as a result of commensalism.

Cooxidation by mixed microbial communities appears to be very important in the degradation of cycloalkanes, since only a few species of bacteria have been shown to use cyclohexane as a sole carbon source (Hornick, Fisher, and Paolini, 1983). Cooxidation changes the cycloalkane to a cycloalkanone, using a variety of oxygenases, such as peroxidase or polyphenoloxidase (Beam and Perry, 1974). A commensalistic symbiosis is then postulated to occur, with the cycloalkanones produced by cooxidation being used as a sole carbon source by a wide range of microorganisms (Donoghue, Griffin, Norris, and Trudgill, 1976). Alkyl side chains on cycloalkanes are degraded by the normal alkane oxidation mechanism before degradation of the cycloalkane itself, depending upon the size of the side chain.

4.1.1.3 Degradation of Alkenes

Unsaturated 1-alkenes are generally oxidized at the saturated end of the molecule by the same mechanism as used for alkanes (Hornick, Fisher, and Paolini, 1983). Some microorganisms, such as the yeast Candida lipolytica, attack at the double bond and convert the alkene into an alkane-1,2-diol. Other minor pathways have been shown to proceed via an epoxide, which eventually is converted into a fatty acid. For similar amounts of degradation, the chain length of 1-alkenes must be longer than the corresponding alkane. Many microorganisms will not grow on 1-alkenes less than C_{12}. Since 2-alkenes are more readily attacked than 1-alkenes, the presence of a terminal methyl group at each end of the molecule appears to make the molecule degradable for more organisms.

4.1.1.4 Degradation of Aromatic Compounds

Five phases can be distinguished for aerobic and anaerobic metabolism of aromatics (Evans, 1977).

1. Entry into the cell--this can be by free diffusion or with specific transport mechanisms

2. Manipulations of the side-chains and formation of substrates for ring-cleavage

3. Ring-cleavage

4. Conversion of the products of ring-cleavage into amphibolic intermediates

5. Utilization of the amphibolic intermediates

The side groups of the ring are first modified by hydroxylation, demethylation, or decarboxylation (which are generally enzymatic reactions) to produce one or two basic molecules, which are then cleaved by the second group of enzymes and further degraded to molecules utilizable by the cell (Hornick, Fisher, and Paolini, 1983). The most common ring cleavage mechanism is the "ortho" pathway. This is followed by a series of enzymatic reactions, with the final products being low molecular weight organic acids and aldehydes that are readily incorporated into the tricarboxylic acid cycle. Polycyclic aromatic hydrocarbons, such as anthracene and phenanthrene, are also degraded by the 'ortho" cleavage pathway. The other major pathway is the "meta" cleavage echanism, where the aromatic ring is cleaved by a dioxygenase to form a keto :id or an aldehydro-acid.

When an alkyl chain is present on a PAH, it is removed by beta-oxidation, it is larger than an ethyl group (Hornick, Fisher, and Paolini, 1983). Ring avage usually can occur when methyl side chains are present; however, when ertain locations on the ring are substituted, the resulting compound is very resistant to degradation (McKenna and Heath, 1976; Gibson, 1976).

In one study, aromatic compounds were found to be susceptible to aerobic, but not anaerobic, biodegradation (Rittmann, Bouwer, Schreiner, and McCarty, 1980). However, halogenated aliphatic compounds evaluated were degradable only under anaerobic conditions and not aerobic conditions (Roberts, McCarty, Reinhard, and Schreiner, 1980). Degradation of chlorinated aromatics only under aerobic conditions suggests the need for mixed-function oxidase systems to bring about dehalogenation and ring cleavage of these compounds (Bitton and Gerba, 1985).

4.1.1.4.1 Degradation of Mononuclear Aromatic Hydrocarbons

Groundwater spiked with benzene, toluene, and xylenes (BTX) was introduced below a shallow water table, and the migration of contaminants was monitored (Barker and Patrick, 1985). The BTX migrated slightly slower than the groundwater due to sorptive retardation. Essentially, all the injected mass of BTX was lost within 434 days due to biodegradation. Rates of mass loss were highest for m- and p-xylene, lower for o-xylene and toluene, and lowest for benzene, which was the only component to persist beyond 270 days. Laboratory biodegradation experiments produced similar rates. However, phenolic and acidic breakdown products observed in laboratory experiments were not found in the field plume. BTX is rapidly degraded under aerobic condition, but persists in conditions of low dissolved oxygen. Washed cell suspensions of P. putida, grown with toluene as a sole source of carbon, were able to oxidize benzene, toluene, and ethylbenzene at equal rates (Gibson, Koch, and Kallio, 1968).

1. Phenol

Phenol is rapidly degraded in aerobically incubated soil (Baker and Mayfield, 1980). Substituted phenols incubated under aerobic conditions are sorbed irreversibly by clays and soils and are transformed into polymerized species (Sawhney and Kozloski, 1984). Phenol can be utilized as the sole carbon source *via* catechol by *Pseudomonas putida*, yeasts, and other organisms. (Ghisalba, 1983). *Acinetobacter calcoaceticus* can degrade phenol and many aromatic compounds (Fewson, 1967).

2. Benzene

The availability of dissolved oxygen is a dominant factor in the biodegradation of benzene (Barker and Patrick, 1985). *Pseudomonas rhodochrous* and *Pseudomonas aeruginosa* metabolize benzene through catechol and *cis*, *cis*-muconic acid (Hou, 1982).

3. Toluene

A major factor in the biodegradation of toluene is the availability of dissolved oxygen (Barker and Patrick, 1985). Turnover time, the amount of time required to remove the concentration of substrate present, for toluene is greater than 10,000 hr; therefore, biodegradation of this compound in aquifers would be expected to be a very slow process (Swindoll, Aelion, Dobbins, Jiang, Long, and Pfaender, 1988).

Toluene can undergo two types of attack: 1) immediate hydroxylation of the benzene nucleus, followed by ring-cleavage or 2) oxidation of the methyl group, followed by hydroxylation and cleavage of the ring (Fewson, 1981). Toluene can be converted to 3-methylcatechol by a *Pseudomonas* sp. and an *Achromobacter* sp (Claus and Walker, 1964).

P. aeruginosa can oxidize toluene (Hou, 1982). Extracts of this organism grown on xylene can also oxidize toluene. The compound is also oxidized by other species of Pseudomonas (e.g., *P. mildenberger*) and *Achromobacter*.

Some soil bacteria (*P. putida*, etc.) can utilize toluene as the sole carbon source (Ghisalba, 1983). More than 90 percent of toluene added to core samples from depths of 1.2, 3.0, and 5.0 m was degraded in one week (McNabb, Smith, and Wilson, 1981). There was no degradation of toluene in material that was autoclaved prior to addition of the compound.

4. Xylene (*o*,*m*,*p*)

A dominant factor in the biodegradation of xylenes is the availability of dissolved oxygen (Barker and Patrick, 1985).

P. putida can directly oxidize the aromatic ring of *p*- and *m*-xylenes (Hou, 1982). These authors were not able to isolate microorganisms that grow on *o*-xylenes. The oxidation of *o*-xylene to *o*-toluene was demonstrated in a *Nocardia* sp. only with the cooxidation technique.

5. Alkylbenzenes

Long-chain alkylbenzenes are oxidized at the terminal methyl group (Hou, 1982). A Nocardia species grows on n-decylbenzene, n-dodecylbenzene, n-octadecylbenzene, n-nonylbenzene, and n-dodecylbenzene. As the alkyl chain length grows and the substituent becomes the major part of the molecule, these compounds are more realistically regarded as substituted alkanes, rather than substituted benzenes. n-Alkylbenzenes are also oxidized by yeasts.

4.1.1.4.2 Degradation of Polynuclear Aromatic Hydrocarbons (PNAs) or Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons are made up of two or more fused benzene rings in linear, angular, or cluster arrangements and contain only carbon and hydrogen (Edwards, 1983). The quantities of PAHs that are formed in nature are very small in comparison with those from anthropogenic sources. Most PAHs are practically insoluble in water. Molecular weights range from 178 to 300. Some are strongly, and some weakly, carcinogenic or mutagenic. The well-known PAHs, such as benzo(a)pyrene (BaP), are innocuous by themselves, but can be biologically activated by enzymes to form epoxides that are carcinogenic and mutagenic (Levin, Wood, Wislocki, Chang, Kapitulnik, Mah, Yagi, Jerina, and Conney, 1978). Conversely, there are some green plants that contain a substance ellagic acid) that can destroy the diol epoxide form of BaP, inactivating its arcinogenic and mutagenic potential (Sayer, Yagi, Wood, Conney, and Jerina, 82). Some of the PAHs of concern are anthracene, benzo(a)pyrene (BaP), nzo(a)anthracene, fluoranthene, phenanthrene, perylene, pyrene, and fluorene. henyl and naphthalene, diaromatics, will be considered as PAHs in this ort.

The toxicity of PAHs to microorganisms is related to their water solubility (Sims and Overcash, 1983). Degradation of some of the less water soluble, such as benzo(a)anthracene and BaP, occurs only when the PAHs are mixed with soil, water, and a substance to stimulate growth of oxygenase-active organisms (Groenewegen and Stolp, 1981). The biotransformation process for PAHs with more than three rings appears to be cometabolism (Sims and Overcash, 1983). Benzo(a)anthracene and pyrene are probably slow to be degraded (Ghisalba, 1983). Generally, PAHs with four or more rings can be degraded by microbes by metabolism via hydroxylation and ring fission. When the soils become acclimated to PAHs, their ability to degrade these compounds is enhanced.

PAH mineralization in sediments is related to the length of incubation time, temperature, molecular size of the substrate and previous exposure to PAH or related contaminants (Sherrill and Sayler, 1982). The capacity of bacteria to degrade BaP increases with BaP content in the soil, and microflora of soil contaminated with BaP are more active in metabolizing BaP than those in "clean" soil (Shabad, Cohan, Ilnitsky, Khesina, Shcherbak, and Smirnov, 1971). On the other hand, a PAH-degrading bacterial population added to various sediment systems did not significantly enhance PAH (naphthalene, phenanthrene, benzo(a)pyrene) mineralization rates (Sherrill and Sayler, 1982).

There is a correlation between PAH contamination and the rate of mineralization of naphthalene (Kerr and Capone, 1986). Rates of PAH mineralization in all environments appear to be primarily controlled by the amount of pollutant present. There is an inverse relationship between ambient concentrations and mineralization/transformation rates (Shiaris and Jambard-Sweet, 1984). The rates are generally not affected by ambient salinity regimes (Kerr and Capone,

1986). However, rates have been shown to be substantially modified by changes in salinity outside ranges normally experienced in the coastal environments tested. The rates are also affected by seasonal fluctuations.

Anthracene, fluoranthene, and phenanthrene are utilized by Pseudomonas spp. as a sole carbon source (Ghisalba, 1983). Napthalene, biphenyl, and BaP can be metabolized by a number of different species of yeast, some of which are reported in high numbers in oil-polluted soils (Cerniglia and Crow, 1981). A filamentous fungus has been noted to degrade BaP and benz(a)anthracene (Dodge and Gibson, 1980; Cerniglia and Gibson, 1979).

Di- and tricyclic aromatic hydrocarbons are extremely insoluble, but it appears that degradation of compounds, such as phenanthrene, may be more rapid in natural environments than was once imagined (Fewson, 1981). The rate is related to previous pollution at the site, the length of time allowed for biodegradation, the temperature, and the molecular weight of the hydrocarbon (Sherill and Sayler, 1980). It has been suggested that naphthalene and phenanthrene are utilized only in the soluble form, since the growth rates of bacteria are related to the solubilities of the hydrocarbons on which they are growing (Wodzinski and Coyle, 1974). Microorganisms, however, could adhere to particles of insoluble substrates (Cox and Williams, 1980). Addition of chemical surfactants could increase the rate of utilization of naphthalene.

1. Alkylnaphthalenes

Degradation of alkylnaphthalenes, by Pseudomonas spp. depends upon the position, number, and type of the substituents on the molecule (Solanas and Pares, 1984). Pure cultures of Pseudomonas spp. preferentially degraded the less substituted aromatics in a light crude oil residue, providing indirect evidence that the organisms are attacking the ring system rather than the alkyl chains. Certain strains of bacteria, notably Pseudomonas spp., metabolize aromatic hydrocarbons with fused benzene rings through cis-dihydrodiol intermediates at different sites of the molecule (Jerina, Selander, Yagi, Wells, Davey, Mahadevan, and Gibson, 1976). It has been, therefore, assumed that alkyl substituents may hinder the initial oxygenative attack of the molecule by the corresponding enzymatic system. However, in some cases, the oxidation rates seem to be enhanced, such as with meta-substituted naphthalenes (Solanas and Pares, 1984). Degradation of naphthalene by Pseudomonas involves the formation of 1,2-dihydroxynaphthalene as the first stable metabolite (Davies and Evans, 1976). Oxidation becomes difficult when a substituent is present in these ortho (1,2) positions, unless it is a methyl group adjacent to the sites of oxidative attack.

2. Naphthalene

A Nocardia sp. can biotransform naphthalene and substituted naphthalenes to their corresponding diols (Hou, 1982). Naphthalene metabolism can be found in many genera from the major fungal taxa.

Candida lipolytica, C. guilliermondii, C. tropicalis, C. maltosa, and Debaryomyces hansenii are able to metabolize this compound (Cerniglia and Crow, 1981). C. lipolytica oxidizes naphthalene to 1-naphthol, 2-naphthol, 4-hydroxy-1-tetralone, and trans-1,2-dihydroxy-1,2-dihydronaphthalene. The primary metabolite is 1-naphthol.

Cunninghamella bainieri oxidizes naphthalene through trans-1,2-dihydroxy-1,2-dihydronaphthalene (Gibson and Mahadevan, 1975). Cunninghamella elegans oxidizes naphthalene by a sequence of reactions resulting in six metabolites: 1-naphthol, 4-hydroxy-1-tetralone, 1,4-naphthoquinone, 1,2-naphthoquinone, 2-naphthol, and trans-1,2-dihydroxy-1,2-dihydronaphthalene.

Naphthalene is oxidized by species of Cunninghamella, Syncephalastrum, and Mucor to 2-naphthol, 4-hydroxy-1-tetralone, trans-naphthalene dihydrodiol, 1,2-naphthoquinone, 1,4-naphthoquinone, and predominantly 1-naphthol (Cerniglia, Hebert, Szaniszlo, and Gibson, 1978). Neurospora crassa, Claviceps paspali, and Psilocybe strains also showed a similar degradative capacity.

Metabolism of naphthalene by Pseudomonas rathonis and Pseudomonas oleovorans with optimal synthesis of salicylic acid was accomplished by addition of 0.4 percent $Al(OH)_3$ (Zajic, 1964). Inorganic boron compounds also increase the yields of salicylic acid.

Algae also oxidize naphthalene (Naval Civil Engineering Laboratory, 1986). Methyl-, dimethyl-, and trimethyl-naphthalenes were more toxic than naphthalene to the freshwater alga, Selenastrum capricornutum, while dibenzofuran, fluorene, phenanthrene, and dibenzothiophene were the most toxic to this organism (Hsieh, Tomson, and Ward, 1980). In general, compounds with higher boiling points were more toxic.

Turnover time for naphthalene was greater than 10,000 hr; therefore, biodegradation of this compound in aquifers would be expected to be a very slow process (Swindoll, Aelion, Dobbins, Jiang, Long, and Pfaender, 1988). However, naphthalene has been reported to be degraded rapidly in aerobic groundwaters contaminated by polynuclear aromatic hydrocarbons or in groundwater near oil and gas beds (Slavnia, 1965). Naphthalene can be slightly sorbed onto sediments (Erlich, Goerlitz, Godsy, and Hult, 1982). It was biodegraded in an aquifer recharged with reclaimed water from wastewater treatment after an initial lag (Roberts, McCarty, Reinhard, and Schreiner, 1980z). These results demonstrate the importance of acclimation of the organisms to the contaminant.

3. Biphenyl

There is evidence that fungi metabolize biphenyl to metabolites similar to those formed by mammalian systems; i.e., the trans-configuration (Hou, 1982). Candida lipolytica, C. guilliermondii, C. tropicalis, C. maltosa, and Debaryomyces hansenii are able to metabolize this compound (Cerniglia and Crow, 1981). C. lipolytica oxidizes biphenyl to 2-, 3-, and 4-hydroxybiphenyl, 4,4'-dihydroxybiphenyl, and 3-methoxy-4-hydroxybiphenyl, with 4-hydroxybiphenyl as the main metabolite.

Strains of Moraxella sp., Pseudomonas sp., and Flavobacterium sp. able to grow on biphenyl were isolated from sewage (Stucki and Alexander, 1987).

4. Benzo(a)pyrene (BaP or BP)

After growth with succinate plus biphenyl, a mutant strain of Beijerinckia contains an enzyme system that can oxidize benzo(a)pyrene to a mixture of vicinal dihydrodiols, mainly cis-9,10-dihydroxy-9,10-dihydrobenzo(a)pyrene (Gibson and Mahadevan, 1975).

Fungi oxidize this compound by a mechanism similar to that observed in mammalian systems (Hou, 1982); e.g., it is oxidized by Cunninghanella elegans to polar products with trans-configuration. Candida lipolytica, C. guilliermondii, C. tropicalis, C. maltosa, and Debaryomyces hansenii are able to metabolize this compound (Cerniglia and Crow, 1981). C. lipolytica oxidizes benzo(a)pyrene to 3-hydroxybenzo(a)pyrene and 9-hydroxybenzo(a)pyrene. Neurospora crassa and Saccharomyces cerevisiae have inducible hydroxylases that can attack this compound (Knackmuss, 1981). Cunninghamella elegans also degrades this compound, with formation of a carcinogen.

Bacillus megaterium strains accumulate BP, which is stored in the cytoplasm and the lipid inclusions of the cells but not in the form of BP-protein complexes (Poglazova, Fedoseeva, Khesina, Meissel, and Shabad, 1967). The chemical remains unaltered in some of the strains but gradually disappears or is reduced in others. When the organisms are grown on a medium that does not contain aromatic hydrocarbons, they eventually lose their ability to destroy BP, but this ability can be restored and further enhanced by adding the compound to the medium.

5. Benzo(a)anthracene (BaA)

A mutant strain of Beijerinckia is able to oxidize benzo(a)anthracene after growing on succinate in the presence of biphenyl (Gibson and Mahadevan, 1975). This compound is metabolized to four dihydrodiols, primarily cis-1,2-dihydroxy-1,2-dihydrobenzo(a)anthracene. Fungi, e.g., Cunninghamella elegans, oxidize this compound to polar products with trans-configuration (Hou, 1982).

6. Anthracene, Phenanthrene, and Pyrene

A Pseudomonas/Alcaligenes sp., an Acinetobacter sp., and an Arthrobacter sp. grew on anthracene or pyrene as the sole carbon source (Stetzenbach and Sinclair, 1986). Pyrene degradation was correlated with the presence of oxygen with no decrease in concentration observed under anaerobic or microaerophilic conditions.

The metabolic pathways for phenanthrene and anthracene are similar to the sequence for naphthalene (Hou, 1982). A Beijerinckia sp. oxidizes these two compounds. An Aeromonas sp. uses an alternative pathway for phenanthrene metabolism.

4.1.1.5 Degradation of Carboxylic Acids including Fatty Acids

P. putida grown on aviation turbine fuel oxidized straight chain fatty acids from acetate (C_2) to palmitic acid (C_{16}) (Williams, Cumins, Gardener, Palmier, and Rubidge, 1981). No odd-numbered fatty acids were detected, indicating that beta-oxidation is the major pathway for fatty acid dissimilation.

Certain species of Pseudomonas oxidize the fatty acids formed from heptane and hexane by classical beta-oxidation (Zajic, 1964).

4.1.1.6 Degradation of Alcohols

Methanol and tertiary butyl alcohol (TBA) may each be present at 5 percent in some gasolines (Novak, Goldsmith, Benoit, and O'Brien, 1985). Methanol is

readily biodegradable in both aerobic and anaerobic environments (Novak, Goldsmith, Benoit, and O'Brien, 1985; Lettinga, DeZeeuw, and Ouborg, 1981). It is degraded in soil samples to 31 m, especially in the saturated region (Novak, Goldsmith, Benoit, and O'Brien, 1985). Many bacteria and yeasts that can catabolize methanol under aerobic or anaerobic conditions and at low and high concentrations have been isolated from deep soil (to 100 ft) (Benoit, Novak, Goldsmith, and Chadduck, 1985).

TBA is more refractory. It is slowly degraded in anaerobic aquifers, with the degradation rate increasing with increasing concentration. At 1 mg/l TBA, it appears the microbial population receives insufficient energy to cause a population increase and utilization rates remain slow. Rates are faster at the highest concentration. Several species that can degrade tertiary butyl alcohol under aerobic conditions have been isolated (Benoit, Novak, Goldsmith, and Chadduck, 1985).

4.1.1.7 Degradation of Alicyclic Hydrocarbons

Pure strains do not grow on these compounds (Hou, 1982). Instead, these materials have been found to be cooxidized by microorganisms growing on other substrates (see Section 5.1.1.2).

n-Alkyl-substituted alicyclic hydrocarbons are more susceptible to microbial attack than the unsubstituted parent compounds; e.g., methylcyclohexane and methylcyclopentane (Hou, 1982).

Cyclohexane and Oxygenates

Complete biodegradation of cyclohexane can be achieved with commensalism by using two organisms--an n-alkane oxidizer that converts cyclohexane into either cyclohexanol or cyclohexanone during growth on the n-alkane and another that can grow on cyclohexanol or cyclohexanone (Beam and Perry, 1974; de Klerk and van der Linden, 1974). Acinetobacter and Nocardia globerula grow rapidly with cyclohexanol as the sole source of carbon. Microbial attack on cyclohexane could be initiated by hydroxylation.

In one experiment, a Pseudomonas strain furnished biotin to allow a Nocardia sp. to grow on cyclohexane (Hou, 1982).

Xanthobacter autotrophicus cannot utilize cyclohexane but can grow with a limited range of substituted cycloakanes, including cyclohexanol and cyclohexanone (Magor, Warburton, Trower, and Griffin, 1986). Another species of Xanthobacter can grow on cyclohexane. Both pathways produce adipic acid.

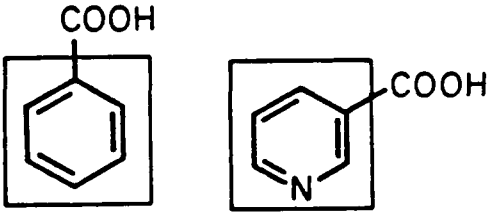
Cyclohexane carboxylic acid and cyclohexane propionic acid are more suitable to microbial growth than cyclohexane acetic acid and cyclohexane butyric acid (Hou, 1982). Effective cleavage of an alicyclic ring occurs only when the side chain contains an odd number of carbon atoms.

4.1.2 Anaerobic Degradation

It is now believed that three major groups of microorganisms are essential for complete mineralization of organic carbon to carbon dioxide and methane in anoxic sites that are without light and are low in electron acceptors other

than carbon dioxide (Berry, Francis, and Bollag, 1987). These three groups are the fermenters, the proton reducers, and the methanogens (Boone and Bryant, 1980; McInerney and Bryant, 1981; McInerney, Bryant, Hespell, and Costerton, 1981).

There are some compounds, most notably the lower molecular weight halogenated hydrocarbons, that will degrade only anaerobically (Environmental Protection Agency, 1985b). Aromatic compounds consisting of either a homocyclic [e.g., benzoate, Figure A.4-2 (a)] or a heterocyclic [e.g., nicotinate, Figure A.4-2 (b)] aromatic nucleus can be metabolized by microorganisms under anaerobic conditions (Berry, Francis, and Bollag, 1987).



(a) (b)

Figure A.4-2. Homocyclic Aromatic "Benzenoid" Nucleus (Enclosed) of Benzoate (a) and Heterocyclic Aromatic "Pyridine" Nucleus (Enclosed) of Nicotinate (b) (Berry, Francis, and Bollag, 1987)

The chemistry of the anaerobic breakdown of aromatics involves an initial ring hydrogenation step (ring reduction followed by a ring hydration ring cleavage reaction sequence (Berry, Francis, and Bollag, 1987). This pathway is believed to be common to all microorganisms involved in benzenoid metabolism, including the denitrifiers, the sulfate reducers, and the fermenters.

4.1.2.1 Anaerobic Respiration

Anaerobic respiration in soil involves biological oxidation-reduction reactions in which inorganic compounds (nitrate, sulfate, and carbonate ions; manganic and ferric ions) serve as the ultimate electron acceptor, instead of molecular oxygen. Nitrate may be used by nitrate reducers and sulfate by sulfate reducers as a terminal electron acceptor (Environmental Protection Agency, 1985b). The organic waste serves as the electron donor or energy source. If oxygen is depleted during decomposition of a particular waste, the system will be dominated by facultative anaerobic bacteria, which are able to adapt from the aerobic to the anaerobic conditions, as necessary. Obligate anaerobes, on the other hand, cannot tolerate oxygen and are inhibited or killed by exposure to it.

Without oxygen, oxygenases are inactive, and only those aromatic compounds with oxygen-containing functional groups (phenols and benzoates) are mineralized (Zeyer, Kuhn, and Schwarzenback, 1986). Under anaerobic

conditions, the aromatic ring may be first reduced to a substituted cyclohexane before hydrolytic ring cleavage. In some cases, removal or modification of a substituent must occur before reduction of the ring. The mechanisms used with aromatic hydrocarbons that have no activating groups to facilitate hydration of the ring are still unknown. However, it does appear that there is an oxidation of the ring in these instances. Apparently, attachment of an oxygen atom to the ring structure facilitates ring catabolism under anoxic conditions (Berry, Francis, and Bollag, 1987).

It appears that many aromatic compounds can be cleaved under strict anaerobic conditions, producing carbon dioxide and methane (Healy and Young, 1979). This decomposition of organic matter to carbon dioxide and methane involves interactions of heterotrophic bacteria and several fastidiously anaerobic bacteria: hydrolytic bacteria that catabolize major components of biomass, such as saccharides, proteins, and lipids; hydrogen-producing, acetogenic bacteria that catabolize products from the activity of the first group, such as fatty acids and neutral end products; homoacetogenic bacteria that catabolize multicarbon compounds to acetic acid; and methanogenic bacteria (Kobayashi and Rittmann, 1982). Both fastidious and facultative anaerobes are important.

If conditions do not favor methanogens, certain classes of organic compounds, such as phenols, cresols, and xylenes, can be degraded by bacteria that respire nitrate or sulfate (Wilson, Leach, Henson, and Jones, 1986). Anaerobic degradation of aromatic hydrocarbons might also be facilitated in nature by the presence of other substrates and oxidants, including oxidized organics, metal-organic compounds, and perhaps even water (Kochi, Tang, and Bernath, 1972).

Petroleum can be microbially degraded anaerobically by the reduction of sulfates and nitrates (Shelton and Hunter, 1975). An alkane dehydrogenase is proposed to be the initial enzyme involved with production of an alkene as the first intermediate compound. The fatty acids produced from the alkanes can be fermented under anaerobic conditions (Rosenfeld, 1947). Alkanes shorter than C_9 can be degraded anaerobically, while some of the longer chain alkanes may be transformed into napthalenes and other polycyclic aromatic hydrocarbons. Anaerobic degradation of aromatic compounds can occur with both the aromatic ring and side chains of substituted aromatic compounds as carbon sources, for many anaerobic microorganisms (Balba and Evans, 1977).

4.1.2.1.1 Denitrification

Microorganisms that carry out nitrate respiratory metabolism (e.g., the denitrifiers) are facultative and appear to prefer oxygen as their electron acceptor (Gottschalk, 1979). Under aerobic conditions, this group of microorganisms uses a wide range of organic compounds as carbon and energy sources. In many instances the same range of organic carbon is used under denitrifying conditions. Active nitrate-respiring microorganisms are found in a variety of anoxic environments, including soils, lakes, rivers, and oceans (Berry, Francis, and Bollag, 1987).

Catabolism of aromatic compounds can occur under anoxic conditions and in the presence of nitrate (Braun and Gibson, 1984). Pseudomonas sp. strain PN-1

can use p-hydroxybenzoate, benzoate, and m-hydroxybenzoate, but not phenol, to grow under both aerobic and nitrate-reducing conditions. This organism appears to have an oxygenase enzyme system that does not require oxygen as an inducer. Some facultative microorganisms retain low levels of oxygenase activity when grown in the presence of aromatic compounds, even under anaerobic conditions, while others do not (Taylor, Campbell, and Chinoy, 1970). A nitrate-respiring P. stutzeri is capable of using phenol as a substrate (Ehrlich, Godsy, Goerlitz, and Hult, 1983).

Microorganisms can degrade o-, m-, and p-xylenes and toluene under denitrifying conditions, such as may occur in lake sediments, sludge digestors, and groundwater infiltration zones from landfills and polluted rivers (Zeyer, Kuhn, and Schwarzenback, 1986; Kuhn, Colberg, Schnoor, Wanner, Zehnder, and Schwarzenbach, 1985).

4.1.2.1.2 Sulfate Reduction

Those microorganisms that carry out dissimilatory sulfate reduction to obtain energy for growth are strict anaerobes (Berry, Francis, and Bollag, 1987). For these bacteria, organic carbon serves as a source of both carbon and energy. Reducible sulfur compounds (e.g., sulfate, thiosulfate) serve as terminal electron acceptors. Dissimilatory sulfate-reducing bacteria (sulfidogens) are most commonly associated with aquatic environments (i.e., marine and freshwater sediments) (Laanbroek and Pfennig, 1981; Lovley and Klug, 1983), although sulfate reducers can also be found in soil.

Organisms, such as Desulfovibrio, utilize sulfate as an electron acceptor, reducing it to sulfide, and by using organic acids as electron donors (Environmental Protection Agency, 1985b). A newly recognized sulfate-reducing organism, Desulfonema magnum, was isolated from marine sediment and is capable of mineralization of various fatty acids and benzoate (but not ethanol, cyclohexane carboxylate, or glucose) to carbon dioxide in the presence of reducible sulfur compounds (Tiedgi, Sexstone, Parkin, Revsbech, and Shelton, 1984).

An interesting syntrophic association between Pseudomonas aeruginosa and Desulfovibrio vulgaris links sulfate respiration to the utilization of benzoate (Balba and Evans, 1980). D. vulgaris seems to produce organic acids, which are used by P.aeruginosa as electron acceptors while it metabolizes the benzoate.

4.1.2.1.3 Methanogenesis

During biodegradation, certain anaerobic bacteria commonly produce short-chain organic acids that can be further broken down to methane, carbon dioxide, and inorganic substances by other bacterial forms (Freeze and Cherry, 1979). Methane bacteria are an example of obligate anaerobes that ferment organic acids to methane. This suggests that such chemicals that enter an anaerobic environment may not be refractory and can possibly be mineralized to carbon dioxide and methane (Healy and Young, 1979). However, organic compounds occurring in a highly reduced state, such as hydrocarbons, are degraded only slowly in an anaerobic environment (Pettyjohn and Hounslow, 1983). In addition, degradation of petroleum hydrocarbons, straight-chain and branched

alkanes, and alkenes, is not possible under methanogenic conditions (Environmental Protection Agency, 1985b).

Bacterial methanogenesis is a process common to many anaerobic environments (Berry, Francis, and Bollag, 1987). This strictly anaerobic process is frequently associated with the decomposition of organic matter in ecosystems, such as anoxic muds and sediments, the rumen and intestinal tract of animals, and anaerobic sewage sludge digesters (Stanier, Doudoroff, and Adelberg, 1970; Lynch and Poole, 1979). Methane bacteria are able to use only a few simple compounds to support growth (Berry, Francis, and Bollag, 1987):

$$CO_2 + 4H_2 \dashrightarrow CH_4 + 2H_2O$$

$$4HCOOH \dashrightarrow CH_4 + 3CO_2 + 2H_2O$$

$$4CH_3OH \dashrightarrow 3CH_4 + CO_2 + 2H_2O$$

$$CH_3COOH \dashrightarrow CH_4 + CO_2$$

The importance of fastidious anaerobic consortia of organisms is illustrated by the types of detoxification reactions known to occur in the animal rumen, the best known of all anaerobic systems (Prins, 1978). These reactions include reductive dechlorination (or dehalogenation), possibly a limiting factor in degradation of certain compounds; nitrosamine degradation, a removal mechanism for a suspected carcinogen; reduction of epoxide groups in various compounds to olefins; reduction of nitro groups, as found in nitrophenol; and breakdown of aromatic structures (Kobayashi and Rittmann, 1982).

Methanogens are an essential component of anaerobic consortia degrading aromatics to methane (Ferry and Wolfe, 1976), but they appear to serve as electron sinks for other organisms rather than themselves attacking the primary substrates (Zeikus, 1977). Acetate and carbon dioxide plus hydrogen are probably the most important substrates for methane bacteria in natural ecosystems (Gottschalk, 1979). Since these organisms can use only simple compounds to support growth, they must rely on syntrophic associations with fermenters, which degrade complex organic compounds (i.e., aromatic compounds) into usable substrates. These associations are generally obligatory and may be similar from one methanogenic habitat to another (Suflita and Miller, 1985). It has been observed that the presence of --Cl or $--NO_2$ groups on phenol can inhibit methane production (Boyd, Shelton, Berry, and Tiedje, 1983).

Many phenolic compounds in a creosote waste were degraded to carbon dioxide and methane by anaerobic bacteria in an aquifer (Ehrlich, Goerlitz, Godsy, and Hult, 1982). However, polynuclear aromatic hydrocarbons, such as naphthalene, were not degraded under the same conditions.

Acetate and formate are important intermediate products in methanogenic fermentation and can indicate the presence of these organisms (McInerney and Bryant, 1981; McInerney, Bryant, Hespell, and Costerton, 1981).

4.1.2.2 Fermentation

During fermentation, obligate and facultative anaerobic bacteria use organic compounds as both electron donors and electron acceptors (*Thibault and* Elliott, 1979). This produces incompletely oxidized organic compounds, such as organic acids and alcohols.

Mixed cultures are often important in the degradative process (Berry, Francis, and Bollag, 1987). Interrelationships exist among various members of the anaerobic community. Neither Pelobacter acidigallici nov. sp. nor Acetobacterium woodii, a demethylating microorganism, was able to degrade the aromatic ring when cultured separately (Bache and Pfennig, 1981; Schink and Pfennig, 1982). However, a coculture completely metabolized syringic acid to acetate and carbon dioxide. In addition, if the isolates were cocultured with Methanosarcina barkeri, any metabolizable aromatic substrate could be completely mineralized to carbon dioxide and methane.

4.1.2.3 Specific Compounds

4.1.2.3.1 Mononuclear Aromatic Hydrocarbons

Laboratory studies confirmed field tests that alkylbenzenes in ground water could be transformed both aerobically and anaerobically (Wilson, Bledsoe, Armstrong, and Sammons, 1986). Anaerobic degradation may be a useful adjunct to the aerobic degradation in heavily contaminated areas of groundwater and soil where the oxygen supply has been depleted.

1. Phenol

Phenol, hydroquinone, and p-cresol are converted to CO_2 and CH_4 under different reducing conditions, and the rate improves with acclimation (Young and Bossert, 1984). Phenolic compounds have been anaerobically biodegraded in near-surface groundwater (Ehrlich, Goerlitz, Godsy, and hult, 1982). Methane and methane-producing bacteria were found only in water from the contaminated zone. However, it has been reported that under anaerobic conditions, transformation of phenols into polymerized species may be inhibited, and they may possibly leach through the soil more readily (Sawhney and Kozloski, 1984).

2. Benzene

Benzene can be biodegraded without the presence of molecular oxygen (Grbic-Galic and Young, 1985; Reinhard, Goodman, and Barker, 1984; Kuhn, Colberg, Schnoor, Wanner, Zehnder, and Schwarzenback, 1985). The anaerobic transformation of benzene might be a fermentation in which the substrates are partially reduced but also partially oxidized (Grbic-Galic and Vogel, 1987). Reduction results in the production of saturated alicyclic rings. Benzene degradation by mixed methanogenic cultures may lead to carbon dioxide and methane with intermediates of phenol, cyclohexane, and propanoic acid. The oxygen for ring hydroxylation might be derived from water. No anaerobic transformation of the compound was observed under methanogenic conditions by enrichment cultures from anaerobic sewage sludge, freshwater sediments, or marine sediments (Schink, 1985). However, after nitrate was injected into a contaminated aquifer, the pollutant was degraded anaerobically (Battermann and Werner, 1984).

Benzene is transformed by methanogenic cultures acclimated to lignin-derived aromatic acids under strictly anaerobic conditions with several intermediates, including demethylation products, aromatic alcohols, aldehydes and acids, cresols, phenol, alicyclic rings, and aliphatic acids (Grbic-Galic and Vogel, 1987). It is ultimately converted to carbon dioxide and methane.

3. Toluene

Toluene can also be biodegraded without the presence of molecular oxygen (Grbic-Galic and Young, 1985; Reinhard, Goodman, and Barker, 1984; Kuhn, Colberg, Schnoor, Wanner, Zehnder, and Schwarzenback, 1985). The anaerobic transformation of toluene may be a fermentation in which the substrates are partly oxidized and partly reduced (Grbic-Galic and Vogel, 1987). Reduction produces saturated alicyclic rings, while oxidation of the methyl group may give a primary product of benzyl alcohol, which, in turn, may be converted to benzaldehyde and benzoic acid.

Microorganisms adapted to growth on m-xylene in the absence of molecular oxygen with nitrate as an electron acceptor are also able to degrade toluene under denitrifying conditions (Zeyer, Kuhn, and Schwarzenback, 1986).

No anaerobic transformation of the compound was observed under methanogenic conditions by enrichment cultures from anaerobic sewage sludge, freshwater sediments, or marine sediments (Schink, 1985). However, after nitrate was injected into a contaminated aquifer, the pollutant was degraded anaerobically (Battermann and Werner, 1984). Methanogenic alluvium from the floodplain of the South Canadian River, which receives leachate from a landfill, showed toluene degradation by an order of magnitude after 11 months (Rees, Wilson, and Wilson, 1985).

Toluene is transformed by methanogenic cultures acclimated to lignin-derived aromatic acids under strictly anaerobic conditions, with intermediates, including demethylation products, aromatic alcohols, aldehydes and acids, cresols, phenol, alicyclic rings, and aliphatic acids (Grbic-Galic and Vogel, 1987). It is ultimately converted to carbon dioxide and methane.

4. Xylene

Xylenes can be biodegraded without the presence of oxygen (Grbic-Galic and Young, 1985; Reinhard, Goodman, and Barker, 1984; Kuhn, Colberg, Schnoor, Wanner, Zehnder, and Schwarzenback, 1985). Up to 0.4 mM 1,3-dimethylbenzene (m-xylene) was rapidly mineralized in a laboratory aquifer column operated in the absence of molecular oxygen with nitrate as an electron acceptor (Zeyer, Kuhn, and Schwarzenback, 1986).

No anaerobic transformation of the compound was observed under methanogenic conditions by enrichment cultures from anaerobic sewage sludge, freshwater sediments, or marine sediments (Schink, 1985). However, after nitrate was injected into a contaminated aquifer, the pollutant was degraded anaerobically (Battermann and Werner, 1984). Other workers also reported that all three xylene isomers could be degraded under anoxic denitrifying conditions in a laboratory column system simulating saturated flow conditions typical for a river water/groundwater infiltration system (Wilson and Rees, 1985). The

three isomers were also preferentially removed over other petroleum products from a methanogenic landfill leachate (Reinhard, Goodman, and Barker, 1984).

m-Xylene can be mineralized in the absence of oxygen by reducing the redox potential, E_h, of the inflowing medium with sulfide to -0.11 V (Zeyer, Kuhn, and Schwarzenback, 1986).

5. Benzoate

Rhodopseudomonas palustris photometabolizes benzoate anaerobically (Atlas and Schofield, 1975). Pseudomonas PN-1 and P. stutzeri grow anaerobically on benzoate-nitrate-mineral salts medium.

4.1.2.3.2 Polycyclic Aromatic Hydrocarbons (PAHs)

1. Naphthalene

No anaerobic transformation of the compound was observed under methanogenic conditions by enrichment cultures from anaerobic sewage sludge, freshwater sediments, or marine sediments (Schink, 1985). Polynuclear aromatic hydrocarbons, such as naphthalene, were not degraded to carbon dioxide and methane by anaerobic bacteria in an aquifer (Ehrlich, Goerlitz, Godsy, and Hult, 1982).

There was no evidence of anaerobic degradation of naphthalene in groundwater samples contaminated by wood creosoting products, although it disappeared at a faster rate in the aquifer than if only dilution were occurring (Ehrlich, Goerlitz, Godsy, and Hult, 1982).

2. Pyrene

Pyrene degradation did not occur under anaerobic or microaerophilic conditions.

4.1.2.3.3 Branched-chain Alkanes and Alkenes

4.1.2.3.4 Straight-chain Aliphatics

4.1.2.3.5 Alcohols

Many bacteria and yeasts that can catabolize methanol under anaerobic conditions and at low and high concentrations have been isolated from soil as deep as 100 ft (Benoit, Novak, Goldsmith, and Chadduck, 1985).

4.1.2.3.6 Alicyclic Hydrocarbons

1. Cyclohexane

A strain of Acinetobacter anitratum utilizes cyclohexane carboxylic acid through 2-oxocyclohexane carboxylic acid (Hou, 1982). Some anaerobic photosynthetic strains, as well as aerobic nonphotosynthetic strains, also metabolize cyclohexane carboxylic acid by this pathway.

4.1.3 End Products

Aerobic breakdown of organic molecules may cause accumulation of organic acid intermediates that reduce soil pH and inhibit biological activity. These effects can be handled with regular reinoculation and use of chemical pH control agents, such as lime. For efficient biodegradation, it is important to have a provision for the removal of toxic wastes and by-products (Texas Research Institute, Inc., 1982). This can be accomplished in two ways: 1) having a diverse microbial population so the by-products are consumed and 2) creating a flow through the system to remove toxins.

Table A.4-7 shows some of the end products formed from the microbial oxidation of hydrocarbons by specific microorganisms (Zajic, 1964).

1-Naphthol is a major product of cyanobacterial metabolism of naphthalene (Cerniglia, van Baalen, and Gibson, 1980a). It is also produced by the filamentous fungus, Cunninghamella elegans, from oxidation of naphthalene (Dagley, 1981). The fate of 1-naphthol in natural environments is of particular interest because it appears to be very toxic (Fewson, 1981). This compound can be totally degraded by some microorganisms (Bollag, Czaplicki, and Minard, 1975), and a soil pseudomonad has been isolated, which can grow on it as a sole source of carbon and energy (Walker, Janes, Spokes, and van Berkum, 1975). 1-Naphthol can also undergo other transformations, such as polymerization by the extracellular enzyme laccase (Fewson, 1981).

The role of phenol oxidases in determining the fate of xenobiotics may have been underestimated (Bollag, Sjoblad, and Minard, 1977). Polymerization reactions and enzymic oxidative coupling are probably important in the covalent bonding of phenolic compounds to soil organic polymers. The degree to which phenols become bound to soil humic molecules as a result of enzymatically mediated oxidative coupling reactions may be affected by substituent groups on the aromatic ring (Berry and Boyd, 1984). For instance, electron donating groups, such as methoxy ($-OCH_3$) (common substituents on lignin-derived polyphenols), would facilitate this reactivity.

It is not completely known at this time what the effect of microbial degradation of complex hydrocarbons may be on the environment (Texas Research Institute, Inc. 1982). It has been established that complete oxidation of many carcinogenic hydrocarbons is not required to render them noncarcinogenic. However, an EPA study reported that, with moderate temperatures and the presence of inorganic nitrogen and phosphorus, naturally occurring freshwater microorganisms are able to form mutagenic biodegradation products from crude oil that are bactericidal to Escherichia coli K-12 (Morrison and Cummings, 1982). The public health significance of this finding is undetermined.

Table A.4-7. Products Formed from the Oxidation of Hydrocarbons by Certain Microorganisms (Zajic, 1964)

Substrate	Microbe	Product
Alkanes	*Pseudomonas* spp[h]	pristane, phytane, sterane, 17 alpha (H), 21 beta (H)-hopane series
	Pseudomonas oleovorans[k]	carboxylic acids
(C_{12} to C_{16})	*Corynebacterium*[a]	ketones, esters of aliphatic acids
(multiple-branched; e.g., pristane)	(*Brevibacterium erythrogenes Corynebacterium* sp.)[a]	succinyl-CoA
(C_{10} to C_{14})	*Corynebacterium*[i]	fatty acids (metabolyzable by beta-oxidation)
Alkenes (C_6 to C_{12})	*Pseudomonas oleovorans*[k]	1,2-epoxides
Benzo(a)anthracene (BaA)	*Cunninghamella elegans*[c]	trans-BA 3,4-, 8,9-, and 10,11-dihydrodiols
	Beijerinck (mutant, acclimated)[g]	dihydrols, primarily c-1,1-dihydroxy-1,2-dihydrobenzo(a)anthracene
Benzo(a)pyrene (BaP, BP)	*Cunninghamella elegans*[b]	trans-7,8-dihydroxy-7,8-dihydro-BP; trans-9,10-dihydroxy-9,10-dihydro-BP; BP-3- and 9-phenols; and BP-1,6- and 3,6-quinones. BP-7,8- and BP-9, 10-diols are further oxidized to metabolites known to be carcinogenic, tumorigenic, and mutagenic to experimental animals
	Candida lipolytica[f]	3-hydroxybenzo(a)pyrene 9-hydroxybenzo(a)pyrene

Table A.4-7. Products Formed from the Oxidation of Hydrocarbons by Certain Microorganisms (Zajic, 1964) (Continued)

Substrate	Microbe	Product
	Beijerinckia (mutant, acclimated)[g]	vicinal dihydrodiols, mainly cis-9,10-dihydroxy-9,10-dihydrobenzo(a)pyrene
	Neurospora crassa[o]	mainly 3-hydroxybenzo-(a)pyrene
	Saccharomyces cerevisiae[p]	7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene, 9-hydroxybenzo(a)-pyrene, 3-hydroxybenzo-(a)pyrene
Biphenyl	*Candida lipolytica*	2-,3-,and 4-hydroxybiphenyl, 4,4'-dihydroxybiphenyl, 3-methoxy-4-hydroxybiphenyl
	Cunninghamella elegans[m]	2-,3-,4-hydroxybiphenyl and 4,4'-dihydroxybiphenyl, glucuronides, sulphates
n-Butane	*M. smegmatis*	2-butanone
Decane	*Corynebacterium*[a]	1-decanol, 1,10-decanediol
Ethane	*Methylosinus trichosporium*	acetone
n-Heptane	*Pseudomonas aeruginosa*	n-hexanoic acid,
Hexadecane	*M. cerificans* *Arthrobacter* sp.[a]	cetyl palmitate ketones
n-Hexane	*M. smegmatis*	2-hexanol, 2-hexanone
Kerosene, heavy naphthas, aromatic naphthas, petroleum pitches, tars, and asphalts	*Fusarium moniliforme*[i]	giberellin
Kerosene	55 strains[i]	amino acids

Table A.4-7. Products Formed from the Oxidation of Hydrocarbons by Certain Microorganisms (Zajic, 1964) (Continued)

Substrate	Microbe	Product
2-Methylhexane	*P. aeruginosa*	2-methylhexanoic acid, 5-methylhexanoic acid
Naphthalene	*Nocardia* sp.	diols of substituted naphthalene
	Cunninghamella elegans	1-naphthol, 2-napththol trans-1,2-dihydroxy-1, 2-dihydronaphthalenes
	Oscillatoria sp. strain JCM[h]	1-naphthol, cis-1,2-dihydroxy-1,2-dihydro-naphthalene, and 4-hydroxy-1-tetralone
	Candida lipolytica[b]	1-naphthol, 2-naphthol, 4-hydroxy-1-tetralone, trans-1,2-dihydroxy-1,2-dihydronaphthalene
	Candida elegans[c]	1-naphthol, 4-hydroxy-1-tetralone, 1,4-naphthoquinone, 1,2-naphthoquinone, 2-naphthol, trans-1,2-dihydroxy-1,2-dihydro-naphthalene
	Cunninghamella elegans, *C. echinulata*, *C. japonica*, *Syncephalastrum* sp., *S. racemosum*, *Mucor* sp., *M. hiemalis*[j]	mainly 1-naphthol; also 2-naphthol, 4-hydroxy-1-tetralone, trans-naphthalene dihydrodiol, 1,2-naphthoquinone, 1,4-naphthoquinone
	Lake sediments[l]	naph-cis-1,2-dihydroxy-1,2-dihydronaphthalene, 1-naphthol, salicylic acid, catechol
Octadecane	*M. cerificans*	octadecyl stearate
Octane	*Pseudomonas*	n-octanol
n-Pentane	*M. smegmatis*	2-pentanone

Table A.4-7. Products Formed from the Oxidation of Hydrocarbons by Certain Microorganisms (Zajic, 1964) (Continued)

Substrate	Microbe	Product
n-Propane	*Mycobacterium smegmatis*	acetone
Phenanthrene		1-hydroxy-2-naphthoic acid[a]
Tetradecane	*Micrococcus cerificans*	myristyl palmitate
Toluene	*Pseudomonas* sp., *Achromobacter* sp.[n]	3-methylcatechol
Tridecane	*Pseudomonas aeruginosa*[a]	tridecane-1-ol, undecan-1-ol
n-Undecane	*Mycobacterium* sp.	undecanoic acid, 1,11 undecandioic acid

Additional references:

a = (Hou, 1982)
b = (Cerniglia and Gibson, 1980)
c = (Dodge and Gibson, 1980)
d = (Cerniglia, Wyss, and Van Baalen, 1980)
e = (Lawlor, Shiaris, and Jambard-Sweet, 1986)
f = (Cerniglia and Crow 1981)
g = (Gibson and Mahadevan, 1975)
h = (Gibson, Koch, and Kallio, 1968)
i = (Zajic, 1964)
j = (Cerniglia, Hebert, Szaniszlo, and Gibson, 1978)
k = (Roberts, Koff, and Karr, 1988)
l = (Heitkamp, Freeman, and Cerniglia, 1987)
m = (Dodge, et al., 1979)
n = (Claus and Walker, 1964)
o = (Lin and Kapoor, 1979)
p = (Wiseman, Lim, and Woods, 1978)

4.2 HEAVY METALS

In the last 200 years, microorganisms have been adapting to the changes in the distribution of elements at the surface of the Earth, as a result of industrialization (Wood and Wang, 1983). Several strategies for resistance to metal ion toxicity have been identified in these organisms:

1. The development of energy-driven efflux pumps that keep toxic element levels low in the interior of the cell (e.g., for Cd(II) and As(V)).

2. Oxidation (e.g., AsO_3^{-2} to AsO_4^{-3}) or reduction (e.g., Hg^{+2} to Hg^0), which can enzymatically and intracellularly convert a more toxic form of an element to a less toxic form

3. Biosynthesis of intracellular polymers that serve as traps for the removal of metal ions from solution (e.g., for Cd, Ca, Ni, and Cu).

4. The binding of metal ions to cell surfaces

5. The precipitation of insoluble metal complexes (e.g., metal sulfides and metal oxides) at cell surfaces

6. Biomethylation and transport through cell membranes by diffusion-controlled processes.

Microorganisms that have short generation times and, consequently, increased evolution rates, have adapted themselves to deal with high concentrations of metal ions. Microorganisms are evolving strategies to maintain low intracellular concentrations of toxic pollutants. Some resist high concentrations through their evolution under extreme environmental conditions (Brock, 1978). Others have achieved resistance to the recently polluted environment through acquisition of extrachromosomal molecules (plasmids) (Silver, 1983).

Metal uptake by a microorganism is metabolism dependent (Hornick, Fisher, and Paolini, 1983). What the organism does not require can be precipitated intracellularly and stored. Microbes are also capable of producing organic compounds, such as citric acid and oxalic acid, which act as binding or chelating agents. Production of hydrogen sulfide by microbes is of great importance in that heavy metals form insoluble sulfides. Both bacteria and yeast have exhibited hydrogen sulfide production and have created a more tolerant environment for more sensitive organisms.

The availability of metal ions for transport into cells is restricted by their abundance and solubility in water (Wood and Wang, 1983). Solubility is greatly influenced by pH, temperature, standard reduction potential (E^0), the presence of competing anions and cations, and the presence of surface-active substances, such as particulates and macromolecules, including proteins, humic acids, and clays.

Both the pH and the E^0 can vary widely from outside the living cell to inside that cell (Wood and Wang, 1983). Most metal ions function as Lewis acids (electron acceptors), but depending upon pH, oxidation state, and complexation, metal complexes also can function as bases. Living cells are not

at equilibrium with the external environment, and, therefore, a kinetic approach to metal ion transport, binding, toxicity, and resistance to toxicity is much more meaningful than a thermodynamic approach.

Metal interactions in biology can be divided into three classes: ions in fast exchange with biological ligands (e.g., Na^{+}, K^{+}, Ca^{+2}, Mg^{+2}, and H^{+}), ions in intermediary exchange with biological ligands (e.g., Fe^{+2} and Mn^{+2}), and ions in slow exchange with biological ligands (e.g., Fe^{+3}, Zn^{+2}, Ni^{+2}, and Cu^{+2}) (Williams, 1983). Living cells have membranes that act as initial barriers to metal ion uptake (Wood and Wang, 1983). Prokaryotes select those ions in fast exchange. Eukaryotes use spatial partitioning of metals. Once the cell buffering capacity for essential metal ions is exceeded, toxicity becomes evident. Toxicity occurs at much lower concentrations for nonessential metals than for essential.

Insoluble metal complexes can be precipitated at the cell surface through the activities of membrane-associated sulfate reductases (Galun, Keller, Malki, Feldstein, Gallun, Siegal, and Siegal, 1983) or through the biosynthesis of oxidizing agents, such as oxygen or hydrogen peroxide (Wood, 1983). The reduction of sulfate to sulfide and the diffusion of oxygen and hydrogen peroxide through the cell membrane provide highly reactive means by which metals can be complexed and precipitated. A green alga, Cyanidium caldarium, can grow in acidic conditions and at high temperatures (Lovelock, 1979). It can remove 68 percent of the iron, 50 percent of the copper, 41 percent of the nickel, 53 percent of the aluminum, and 76 percent of the chromium from solution. Anaerobic cultures growing in the dark produce hydrogen sulfide gas for sulfide precipitation of metals.

Some microorganisms can use biomethylation to eliminate heavy metals, such as mercury and tin, and metalloids, such as arsenic and selenium (Wood and Wang, 1983). The synthesis of less polar organometallic compounds from polar inorganic ions has certain advantages for cellular elimination by diffusion-controlled processes (Wood, Cheh, Dizikes, Ridley, Rackow, ad Lakowicz, 1978). Mechanisms for B_{12}-dependent synthesis of metal alkyls (requiring the presence of vitamin B_{12}) have been discovered for the metals Hg, Pb, Tl, Pd, Pt, Au, Sn, and Cr and for the metalloids As and Se (Craig and Wood, 1981).

Heavy metal contaminants can be altered by transformation to volatile forms (Arthur D. Little, Inc., 1976). Soil reactions can transform them to less toxic forms or make them unavailable to plants. Modifications in soil conditions can promote transformation of mercury and arsenic (e.g., increasing organic material, moisture, temperature, and pH of the soil for mercury and decreasing the oxygen level for arsenic).

There is a possible sequence of activating measures to increase transformation of these metals (Arthur D. Little, Inc., 1976). The first steps would be to add organic material, decrease oxygen, increase temperature, and decrease pH. Logistically, lowering the pH should come first, since ferrous sulfate should be added before irrigation begins. The organic matter should be incorporated into the soil next, immediately preceding irrigation; this will help produce anaerobic conditions. A PVC sheeting can then be applied. These measures should be kept in operation for about two to three years. Monitoring will indicate the actual time required. After the level of one toxicant is acceptable, conditions can be changed to transform and remove another.

Finally, efforts must be made to decrease the availability of heavy metals and other undegradable toxicants left in in the soil; e.g., increasing the pH of the soil with an application of limestone. Leaching of metals must be prevented throughout the treatment process.

Figure A.4-3 presents a possible sequence for soil manipulation

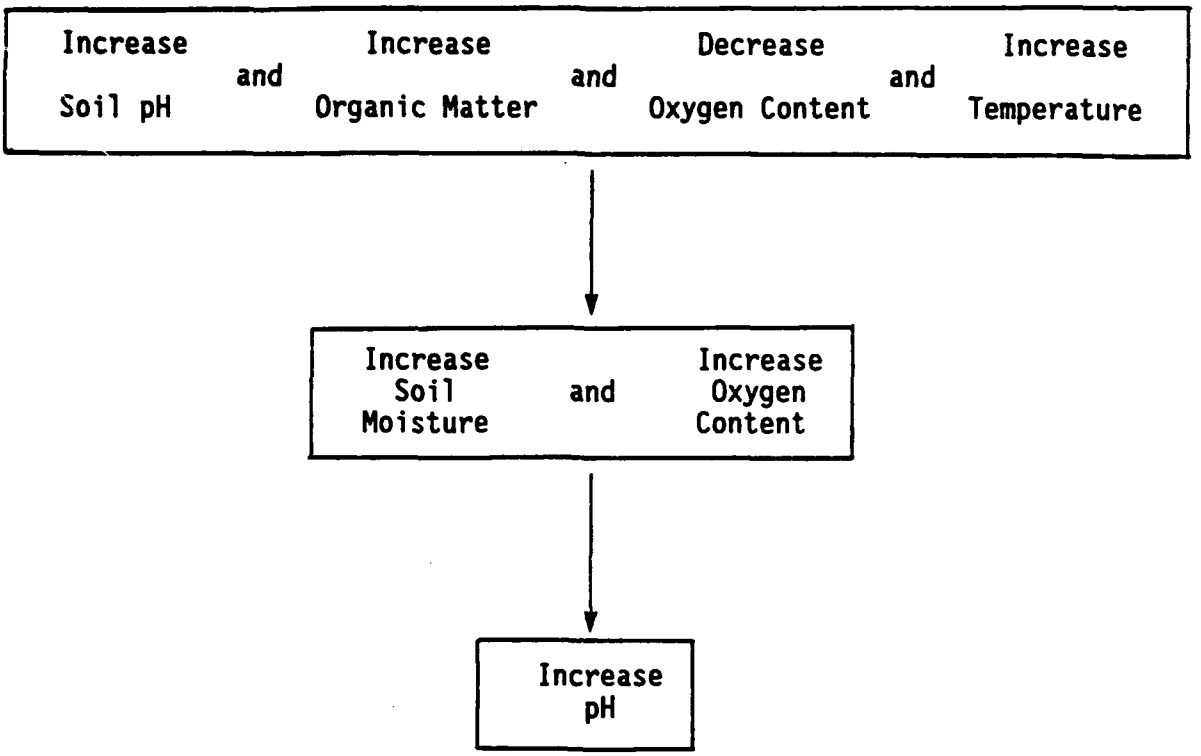


Figure A.4-3. Possible Sequencing of Soil Manipulation (Arthur D. Little, Inc., 1976).

Bacterial resistance to heavy metals in the environment can result in bioaccumulation, biotransformation, changes in ecological diversity, and coselection of resistance factors for antibiotics (Sterritt and Lester, 1980). Microorganisms can be used for the removal of heavy metals from industrial effluents and as indicator organisms in bioassays (Anderson and Abdelghani, 1980). A simple, rapid method for the determination of bacterial resistance to a wide range of metals has been developed through modification of the antibiotic susceptibility test (Thompson and Watling, 1983).

Table A.4-8 lists bacteria, fungi, and algae; the metals they can remove; and the methods they use for removal (Monroe, 1985).

Table A.4-8. Microbial Mechanisms for Metal Extracting/Concentrating/Recovery (Monroe, 1985)

Microorganism	Metal Removed	Method
Thiobacillus, Sulfolobus	Iron, sulfur	Oxidation
Sphaerotilus, Leptothrix, Hyphomicrobrium, Gallionella	Iron, manganese	Oxidation
Spirogyra, Oscillatoria, Rhizoclonium, Chara	Molybdenum, selenium, uranium, radium	Oxidation
Desulfovibrio spp.	Mercury, lead	Reduction
Scenedesmus, Synechococcus, Oscillatoria, Chlamydomonas, Euglena	Nickel	Surface ion-exchange
Saccharomyces cerevisiae, Rhizopus arrhizus	Uranium, cesium, radium	Surface ion-exchange
Penicillium digitatum	Uranium	Surface ion-exchange
Ustilago sphaerogena	Iron	Surface chelation
Aspergillus niger	Aluminum	Surface chelation
Cynanidium caldarium	Iron, copper, nickel, aluminum, chromium	Surface precipitation
Staphylococcus aureus, Escherichia coli	Cadmium, zinc, arsenate, arsenite, antimony	Chemosmotic efflux
Pseudomonas aeruginosa	Uranium, cesium, radium	Intracellular trap
Synechococcus	Nickel, copper, cadmium	Intracellular trap
Clostridium cochlearium	Mercury	Biomethylation
Pseudomonas spp.	Tin	Biomethylation

4.2.1 Specific Elements

The following are the elements present in the fuels addressed in this review and their potential for transformation by soil microbes (JRB Associates, Inc., 1984b):

4.2.1.1 Arsenic (As)

Arsenic is apparently oxidized by aerobic heterotrophic organisms, and many heterotrophs can also reduce arsenate (Alexander, 1977). Methylation of arsenicals is an important process in soils, and trimethylarsine is an important gaseous product (Woolson, 1977). Soil contaminated with arsenic must be managed to minimize volatilization through microbial reduction. Addition of organic material and maintenance of aerobic conditions can help stimulate the oxidation of arsenite to arsenate (Sims and Bass, 1984). Further treatment with ferrous sulfate will form highly insoluble $FeAsSO_4$.

Resistance of microbes to arsenic occurs through the evolution of cellular exclusion mechanisms (Wood and Wang, 1983). Resistance in Staphylococcus aureus and Escherichia coli to arsenate and arsenite is induced by an operonlike system (Novick, Murphy, Gryczan, Baron, and Edellman, 1979). An operon is a DNA region that codes for several enzymes in a reaction pathway.

Arsenic behaves much like phosphorus in soils in that its adsorption increases as iron oxide content increases, and that iron and aluminum hydrous oxides specifically adsorb the metal (Hornick, 1983). Arsenate, however, can be reduced to arsenite, but arsenate is the most common form in soils. Generally, both forms are strongly retained in soils.

4.2.1.2 Cadmium (Cd)

Growth of soil bacteria is retarded by cadmium, and the soil microbial community structure is affected (JRB Associates, Inc., 1984b). However, bacterial populations in contaminated sites have been found to be able to adapt to the heavy metal contamination (Tripp, Barkay, and Olson, 1983). Soil microbial biomass may contribute to the soil cadmium-binding capacity and affect cadmium availability, with dead cells sorbing more cadmium than live cells (Kurek, Czaban, and Bollag, 1982). Since cadmium exists in nature only in the valence state of +2, microbial oxidation or reduction of this element is unlikely.

Intracellular traps can be biosynthesized as a temporary measure for organisms to remove metal ions (e.g., synthesis of metallothionen and removal of cadmium by this sulfhydryl-containing protein (Williams, 1981b), to prevent metals from reaching toxic levels (Wood and Wang, 1983). Mutants with intracellular trapping mechanisms tend to bioconcentrate the toxic metal intracellularly to about 200 times over the external concentration. This strategy works well for some organisms but is not as effective as the extracellular binding or precipitation of metals.

Cellular exclusion mechanisms are also responsible for resistance of microorganisms to cadmium (Wood and Wang, 1983). This resistance is mediated by a plasmid. Resistant cells of Staphylococcus aureus have a very efficient

chemosmotic efflux system specific for Cd^{+2} ions, as a result of two separate plasmid genes.

Cadmium is complexed by organic matter, oxides of iron and manganese, and chlorides (Chaney and Hornick, 1978). In alkaline, low organic matter, sandy soils, precipitation of cadmium compounds occurs. The soil pH is the most important factor governing cadmium solubility and resultant availability, with the solubility increasing as pH decreases.

4.2.1.3 Chromium (Cr)

Chromium should be amenable to oxidation or reduction by microbes, since it commonly exists in two oxidation states, Cr III and Cr VI (Zajic, 1969). Chromium VI is more toxic and mutagenic than chromium III (Ross, Sjogren, and Bartlett, 1981). Gram negative bacteria are also more sensitive to chromium VI than gram positive organisms. It should not be assumed that chromium III is harmless to the soil microflora at high levels.

The most soluble, mobile, and toxic form of chromium in soils occurs in the hexavalent state as chromate or dichromate (Hornick, 1983). When aerobic conditions exist, the hexavalent form is rapidly reduced to trivalent chromium, which forms insoluble hydroxides and oxides and is unable to leach. Liming soil to pH 6.5 with the presence of an alkaline oil waste in the soil will maintain the soil pH near neutral (Hornick, Fisher, and Paolini, 1983). A near neutral soil pH will also prohibit the formation of dichromates (Hornick, 83).

Solid phase chromium should not be readily mobilized during the retention edge slurries in disposal areas, possibly because of the slow oxidation of ed chromium hydroxide (Lu and Chen, 1977). Little chromium is released dispersed sediments under oxidizing conditions. Reduced chromium (Cr III) enerally highly insoluble at pH values above 5.5, unless complexed with ble organic compounds. Chromium has also not been noted to oxidize to more ble chromium (VI) forms under short-term oxidizing conditions.

The green alga, Cyanidium caldarium may be effective for selective removal hromium from polluted wastewaters (Wood and Wang, 1983).

.1.4 Iron (Fe)

The concentration of free metal ions can be controlled by the biosynthesis ligands in the form of small molecules with high stability constants, such with the removal of iron (Wood and Wang, 1983). The cell may expend energy pump the metal ion out of the cell, it may synthesize ligands that bind tals strongly at the cell surface, or it may use the activities of surface- und enzymes to precipitate metals extracellularly.

.2.1.5 Lead (Pb)

Inorganic lead is toxic to a broad range of microorganisms, including yanobacteria, marine algae, fungi, and protozoa (JRB Associates, Inc., 1984b). ead and its compounds also affect microbial activity in soil, including nhibition of nitrogen mineralization, stimulation of nitrification, and the synthesis of soil enzymes. Species diversity is lower in lead-contaminated

soils. A low pH of 5 or 6 increases the toxicity of lead, while higher pH and other abiotic factors (phosphate and carbonate ions, clay minerals, particulate humic acid, and soluble organics) reduce toxicity. Lead can be methylated by some microbes, thereby increasing its volatility and potential loss from the soil.

Lead solubility is determined by the amount of sulfate, phosphate, hydroxides, carbonates, and organic matter present in a soil system (Hornick, 1983). The formation of lead sulfate at very low pH, lead phosphate and hydroxides at intermediate pH, and lead carbonates at a calcareous pH limits the mobility of lead in soils. As the soil pH and available phosphorus in a soil decrease, soluble lead increases.

4.2.1.6 Mercury (Hg)

Microbes oxidize, reduce, methylate, and demethylate mercury (JRB Associates, Inc., 1984b). Serratia marcescens had the highest rate of mercury transformation at pH 8 over the range tested (Mason, Anderson, and Shariat, 1979). Both aerobic and anaerobic heterotrophic bacteria were resistant to 14 ppm Hg^{+2} (Callister and Winfrey, 1983). Mercury-resistant populations of oil-degrading bacteria have been isolated (Walker and Colwell, 1974a). Petroleum biodegradation proceeded when mercury in the soil was present in the low ppm concentration range, but was absent at 85 ppm (Walker and Colwell, 1976a). Mercury methylation was highest in anaerobically incubated surface sediments (Callister and Winfrey, 1983). Sediment-bound mercury remained available for methylation over seven days.

Mercury methylation and demethylation are usually ascribed to different bacteria; however, the anaerobic Clostridium cochlearium T-2 was found to acquire demethylating capabilities, in addition to its methylating (Pan-Hou, Hosono, and Imura, 1980). This trait is probably on a plasmid. Mercury resistance is an inducible trait, and the genetic material coding for it is transposable (Silver and Kinscherf, 1982). Biomethylation of Hg(II) salts to CH_3Hg^+ by a B_{12}-dependent strain of C. cochlearium allows detoxification and gives the organism an advantage in mercury-contaminated systems (Wood and Wang, 1983). Mercuric and organomercurial strains of bacteria have been isolated from a variety of ecosystems, such as soil, water, and marine sediments (Friello and Chakrabarty, 1980; Vonk and Sjipesteijn, 1973). These organisms catalyze both the forward and reverse conversion of CH_3Hg^+ to Hg^{+2} and then to Hg^0 (Wood, 1983).

Mercury reacts in soils with chlorides and sulfur to form insoluble HgS, $HgCl_3$, and $HgCl_4^{-2}$ (Hornick, 1983). Mercury can be chelated by organic matter as $HgCl_4^{-2}$ or can be absorbed by sesquioxide surfaces (CAST, 1976). Through chemical and microbial degradation, mercury can be volatilized or associated with clay particles and organic matter. Mercury is not very mobile in the soil profile due to its strong sorption reactions with soil constituents.

An enzyme capable of metabolizing phenylmercuric acetate has been isolated from soil microorganisms (Tonomura and Kanzaki, 1969). The enzyme(s) is capable of cleaving the carbon-mercury bond and requires both a sulfhydryl compound and NADH to carry out the reaction.

4.2.1.7 Nickel (Ni)

Nickel inhibits soil nitrification, carbon mineralization, and the activities of acid and alkaline phosphatase and arylsulfatase (JRB Associates, Inc., 1984b). Toxicity to fungi is reduced by the presence of clay or an increase in pH to 7.0 (Babich and Stotzky, 1983a; 1983b). Survival of certain bacteria and yeasts was improved by raising the pH from 4.9 to 7.7. The presence of ions such as Mg^{+2}, Zn^{+2}, S^{-2}, PO^{-3}, and PO_4; alkaline pH; and type and amount of clay minerals greatly reduce the microbial toxicity of nickel. However, other ions (e.g., potassium, sodium, calcium, and iron), amino acids, tryptone, casamino acids, yeast extract, and chelating agents (citrate, EDTA, DPA, NTA) do not reduce Ni toxicity.

Both nickel binding and nickel toxicity are very pH dependent (Wood and Wang, 1983). The optimum pH for binding is between 8 and 8.5. Orientation of ligands at the cell surface must be important, since only surface-active substances, such as humic acids, could compete effectively for nickel binding (Galun, Keller, Malki, Feldstein, Galun, Siegal, and Siegal, 1983).

Nickel availability in soils is governed by iron and manganese hydrous oxides and by organic chelates that complex nickel less strongly than copper (CAST, 1976). Nickel differs from copper and zinc in that it is more available from organic sources than inorganic sources. Acid soils increase the solubility of nickel in the soil solution.

Green algae are much more resistant to high concentrations of Ni^{+2} than are the blue-green algae (Wood and Wang, 1983). Cyanobacteria and brown and green algae all bioconcentrate nickel (Fuge, 1973; Karata, Yoichi, and Fumio, 1980; Ballester and Castelvi, 1980; Hirschberg, Skane, and Thorsby, 1977). The former are more sensitive to nickel toxicity than the green algae, indicating different transport mechanisms for prokaryotes and eukaryotes (Galun, Keller, Malki, Feldstein, Galun, Siegal, and Siegal, 1983). Nickel-tolerant mutants of the cyanobacterium, Synechoccus, can tolerate up to 20×10^{-5} M nickel sulfate by synthesizing an intracellular polymer that removes nickel from solution (Simon and Weathers 1976). This intracellular trap prevents nickel toxicity. The green alga, C. caldarium, may be effective in treating waters polluted with nickel, so effluents can meet Federal standards (Wood and Wang, 1983).

4.2.1.8 Selenium (Se)

Normal soils contain between 0.1 and 2.0 ug/g selenium (Girling, 1984). Selenium behaves similarly to sulfur in the soil solution, existing as selenates and selenite (Hornick, 1983). Selenate, the predominant form in alkaline soils, is quite soluble as $CaSeO_4$ and can move readily in these soils. Addition of lime will increase the availability of selenium (Gissel-Nielsen, 1971).

Most of the selenium in the soil is biologically unavailable (Girling, 1984). Some bacteria and fungi reduce biologically available selenium to elemental insoluble forms, while others produce volatile organic forms of selenium that are lost to the atmosphere. Some bacteria are able to oxidize colloidal selenium to selenate or selenite so it becomes biologically available. Soil microflora are capable of several transformations of selenium, such as oxidation, reduction, and methylation (JRB Associates, Inc., 1984b).

The oxidized form, selenate, is very toxic (Alexander, 1977). Methylation is greatly accelerated when a readily available carbon source, such as glucose, is added to the soil.

Selenium salts can be biologically methylated to volatile organic products (Challenger and North, 1933). The fungus, Scopulariopsis brevicaulis, can produce dimethylselenide from inorganic selenite or selenate. Eleven strains of fungi have been found capable of producing dimethylselenide, including strains of Penicillium, Fusarium, Cephalosporium, and Scopulariopsis (Barkes and Fleming, 1974). Strains of Bacillus spp. are able to oxidize around 1.5 percent of the total selenium added to selenite and trace amounts of selenate (Shrift, 1973). Inorganic selenium can be reduced to elemental selenium. Isolates of bacteria, fungi, and actinomycetes from soils containing high selenium contain as much as 0.18 percent (dry wt.) selenium inside their cells (Koval'skii, 1968). Their resistance to the high selenium levels depends upon their ability to reduce the soil selenium to the biologically inert elemental form.

Waste material containing significant amounts of selenium may present environmental problems if the selenium can be transformed into a form that is biologically available (Girling, 1984).

4.2.1.9 Silver (Ag)

Silver is known to be bacteriocidal (Woodward, 1963); however, soil treated with 100 ppm Ag had about twice as many Ag^+-reducing bacteria than untreated soil (Klein and Molise, 1975; Sokol and Klein, 1975).

Several bacteria have been found that precipitate silver as Ag_2S at the cell surface (Wood and Wang, 1983).

SECTION 5

ENHANCEMENT OF BIODEGRADATION

5.1 OPTIMIZATION OF SOIL BIODEGRADATION

Some of the information in this section may duplicate material covered in Section 5.2, Optimization of Groundwater Biodegradation, and Section 5.3, Optimization of Freshwater, Estaurine, and Marine Biodegradation; however, it is presented here under a separate heading, with other related information, to accommodate those readers who may specifically wish to address treatment of soil contamination only.

5.1.1 Biological Enhancement

5.1.1.1 Seeding of Microorganisms

The seeding of microorganisms has been used in a number of different environments to degrade organics (Environmental Protection Agency, 1985b). Microbial seeding does not appear to be very effective in reducing oil contamination in the sea (Atlas and Bartha, 1973e; Gutnick and Rosenberg, 1979; McDowell, Bourgeois, and Zitrides, 1980), but it has been argued that seeding and nutrient supplementation would work in contained environments like oil tankers (Gutnick and Rosenberg, 1979).

Seeding with hydrocarbonoclastic microorganisms appears to be a special application to severely environmentally distressed soil (Cook and Westlake, 1974), such as in Arctic or near-Arctic climates, where the activity and growth rate of indigenous organisms may be limited (Cook and Westlake, 1974; Hunt, et al., 1973). When an addition of 10^4 microorganisms/g dry soil was tested in such an environment (with addition of N and P and adjustment of the pH to 7), microbial activity over the controls was increased by at least a factor of four in 40 days.

However, there have been varying degrees of success with seeding microorganisms onto petroleum-contaminated soils (Knox, Canter, Kincannon, Stover, and Ward, 1968). Other examples have not been as successful. Addition of 10^8 cells/g soil of two hydrocarbon-degrading isolates did not significantly influence oil concentrations (Lehtomakei and Niemela, 1975), and application of 10^6 oil-utilizing bacteria/cm^2 resulted in only a slight additional degradation of the C_{20} to C_{25} group of n-saturated compounds (Jobson, Cook, and Westlake, 1972). The seed organisms in the latter case were species of Flavobacterium and Cytophaga (41 percent), Pseudomonas (34 percent), Xanthomonas (10 percent), Alcaligenes (9 percent), and Arthrobacter (5 percent). Even when oil-degrading bacteria were added to soil of the boreal region of the arctic, there was no increase in the changes of the recovered oil; however, this may have been due to insufficient application (Westlake, Jobson, and Cook, 1978).

Microorganisms able to degrade organic pollutants in culture sometimes may fail to function when inoculated into natural environments because the concentration of the hydrocarbon in nature may be too low to support growth or because the organisms may be susceptible to toxins or predators in the

environments (Alexander, 1986). The organisms may use other organic compounds in preference to the pollutant, or they may be unable to move through the soil to sites containing the chemical.

Use of microorganisms from sewage effluent is a low-cost, fairly effective method for the removal of water-soluble biodegradable organics (Wentsel, et al., 1981). Formaldehyde was successfully removed at levels less than 2000 ppm with such a population mixed with a Pseudomonas sp. Attempts to acclimate an activated sludge culture to groundwater contaminated by several priority pollutants and at least 70 other organics were only minimally successful (Shuckrow and Pajak, 1981). However, coupling an activated sludge process with treatment by granular activated carbon proved beneficial. Microbial growth on the activated carbon may also play a role in the removal of contaminants (Werner, 1982).

Generally, the organisms may be applied in liquid suspension or with a solid carrier (Sims and Bass, 1984). Run-on and run-off controls may be necessary. The potential achievable level of treatment is high. Nevertheless, relatively long periods of time may be required to complete treatment, and excessive precipitation may wash out the inoculum, necessitating retreatment.

Seed microorganisms would have to be freeze dried or frozen to maintain viability (Atlas, 1977). Growing cultures are metabolically active for immediate biodegradation, but would require large volumes and would be difficult to transport to a location. Frequent transferring also raises the danger of contamination or mutation. Mixing of organisms should not be done until just prior to application to prevent competition within the mixture. Freeze-dried or frozen cultures could be mixed well in advance without this problem. Freeze-dried cultures would occupy small volumes for easy transport. However, freeze-dried cultures are quite expensive to prepare and take some time to become metabolically active again, especially in the suboptimal environmental condition of the environments in which they would be used. An alternative method would be to store the culture in a freeze-dried state and then initiate growth in a laboratory just before actually seeding. Then the organisms can be added in the appropriate concentrations to the environment.

The size of the inoculum required depends largely upon the size of the spill, how it is dispersed, and on the growth rate of the seed microorganisms. Some workers report that the size of the inoculum is of little importance, as long as a minimal inoculation of the oil occurs (Miget, 1973). Of greater importance is whether the oil will support the growth of the seed organism in the given environment. However, other researchers concluded that too low a level of application (10^6 bacterial cells/cm^2) was responsible for the only slight increase in the rate of oil utilization (C_{20} to C_{25}) obtained after seeding, rather than the inability of the added bacteria to survive under the natural field conditions (Jobson, McLaughlin, Cook, and Westlake, 1974).

Migration of microorganisms through soil can occur via two processes: convective transport and molecular "diffusion" (Ahlert and Kosson, 1983). Convective transport involves addition of significant quantities of an aqueous nutrient feed solution that causes movement of organisms with the feed and distribution throughout the soil column. Microbiological "diffusion" is analogous to molecular or surface diffusion. It occurs as a result of the life/death cycle and the natural movements of microorganisms. If the selective

organisms added to in situ systems have to depend only on growth to diffuse through the subsurface, it will take too long for bioaugmentation to be cost-effective (Kobayashi and Rittmann, 1982). However, the organisms can be spread through the soil more rapidly by convective transport when the soil is flooded with nutrient solution and the water is continuously pumped from the aquifer, establishing a closed path that pulls the liquid and organisms through the soil.

5.1.1.1.1 Commensals

In a process that uses both cooxidation and commensalism, complete biodegradation of cyclohexane can be achieved in a two-step process using two organisms--an n-alkane oxidizer, such as a pseudomonad, that first converts cyclohexane into either cyclohexanol or cyclohexanone during growth on the n-alkane (e.g., n-heptane) and another strain (e.g., a pseudomonad) that can grow on cyclohexanol or cyclohexanone (de Klerk and van der Linden, 1974; Donoghue, Griffin, Norris, and Trudgill, 1976). The same general results have been reported for the oxidation of other unsubstituted cycloparaffinic hydrocarbons (Beam and Perry, 1974).

5.1.1.1.2 Acclimated Microorganisms

Acclimated microbes and nutrients can be employed to stimulate degradation (Quince and Gardner). However, before a cleanup system employing acclimated bacteria can be implemented, a laboratory investigation of the kinetics of biodegradation for the acclimated bacteria, the potential for inhibition under various conditions, the oxygen and nutrient requirements, and the effects of temperature should be evaluated (Sommers, Gilmore, Wildung, and Beck, 1981). Emulsifiers may be necessary to increase the solubility of the contaminant. The hydrogeologic data that are needed are formation porosity, hydraulic gradient, depth to water, permeability, groundwater velocity and direction, and recharge/discharge information .

Many systems using acclimated bacteria recharge the effluent from biological treatment to the aquifer to create a closed loop of recovery, treatment, and recharge (Quince and Gardner, 1982). This flushes contaminants out of the soil rapidly and establishes hydrodynamic control separating the contaminated zone from the rest of the aquifer.

The systems that have used acclimated bacteria to restore contaminated aquifers typically have relied on biological wastewater treatment techniques, such as activated sludge, aerated lagoons, trickling filters, aerobic digestion, composting, and waste stabilization (Lee and Ward, 1985). These bacteria can be added to the aquifer and can act in situ to degrade the contaminant. The recharge water can be adjusted to provide optimal conditions for the growth of the acclimated bacteria and of the indigenous populations, before returning it to the soil. The bacteria, air, and nutrients can be injected into the subsurface after levels of organics have been reduced by treatment in clarifiers and through air stripping (Quince and Gardner, 1982) or by treatment with clarification, adsorption onto granular activated carbon, air stripping, and then recharge (Ohneck and Gardner, 1982). However, an experiment showed that indigenous bacteria were able to metabolize contaminants at the same or greater rates than a hydrocarbon-degrading bacteria inoculum.

Acclimated organisms have been used effectively in remedial actions for cleaning up contaminated aquifers (Lee and Ward, 1984; Quince and Gardner, 1982). An acrylonitrile spill was handled by removing the groundwater, treating it with mutant bacteria in a small reactor, and recharging it (Polybac Corporation, 1983). Acclimated organisms were used to clean up contaminated groundwater at a site where ethylene glycol and propyl acetate had been spilled (Lee and Ward, 1985). Initial treatment by air stripping and clarification was followed by addition of acclimated bacteria, nutrients, and air to the groundwater. The total organic carbon in the water was reduced from 40,000 to 1 ppm. In another instance, groundwater from a highly polluted aquifer near a hazardous waste dump site was treated in a batch study with organisms from several wastewater treatment systems receiving the organics found in the groundwater (phenols, creosols, dichlorobenzene, and others). After three weeks of acclimation and stabilization, these organisms were able to reduce the organic levels by more than 80 percent within 24 hr.

5.1.1.1.3 Mutant Microorganisms

Genetically engineered microorganisms have been used in cases of environmental contamination. In two instances of mutant bacteria being added to groundwater contaminated by acrylonitrile, levels of the contaminant fell from 1000 ppm to the limits of detection in one month in one case (Walton and Dobbs, 1980) and from 100 ppm to 1 ppm in three months, in the other (Polybac Corporation, 1983). However, in both cases, there was no conclusive evidence that the added organisms contributed more to the degradation in the field than the normal flora of the site.

Genetic Engineering

For those compounds that are recalcitrant to attack by natural microbes, there is the theoretical possibility of constructing strains that can degrade them by the introduction into one bacterium of a number of enzyme activities from different bacteria, which under natural circumstances might have little or no chance to exchange genetic information (Williams, 1981a).

Microorganisms that are potentially useful for treating organic wastes must first be isolated or enriched from natural microbial communities in the environment (Pierce, 1982a). Genetic manipulation of these strains requires extensive knowledge of the biochemistry of the microbial pathway under investigation (Leisinger, 1983). Information of the rate-limiting steps in the degradative pathways, on the substrate specificities of the relevant enzymes, and on the types of regulatory mechanisms involved in gene expression are prerequisites for a rational approach in strain construction. The diversity of substrate utilization of the organism must be determined; growth may be possible on more organic compounds than were used for initial isolation (Pierce, 1982a). This allows the construction of a profile of catabolic activity, which facilitates the physiological, enzymatic, and genetic characterization needed for rational improvement of the strain. Those with high substrate diversity have complex regulatory mechanisms with considerable interaction between plasmid and chromosomal genes. Appropriate hosts and cloning vectors must be carefully selected. The natural origin, as opposed to laboratory or clinical strains, of the isolated organisms may be of value when the modified strains are then used in waste treatment.

Catabolic plasmids in microorganisms occur naturally and some of the common and well-studied catabolic plasmids are listed in Table A.5-1 (Jain and Sayler, 1987).

Table A.5-1. Some Catabolic Pathways Encoded by Naturally Occurring Plasmids in Microorganisms (Jain and Sayler, 1987)

Growth Substrate	Plasmid	Microorganism
Toluene, m-Xylene, p-Xylene	TOL (pWWO); pDK1	*Pseudomonas putida*
Naphthalene	NAH7	*P. putida*
Salicylate	SAL	*P. putida*
Camphor	CAM	*P. putida*
n-Octane	OCT	*P. putida*
4-Chlorobiphenyl	pKF1; pSS50	*Acinetobacter* sp. P6; *Alcaligenes* sp. A5
3-Chlorobenzoate	pAC25	*Pseudomonas* sp. B13
2,4-Dichlorophenoxyacetate	pJP1	*A. paradoxus*
Styrene	—	*P. fluorescens*

The degradation potential of microbes can be altered by genetic manipulation (Zitrides, 1978; McDowell, Bourgeois, and Zitrides, 1980). Radiation can be used to increase the genetic variability of an adapted microbial population. Selected strains of bacteria are chosen for their known ability to degrade similar compounds and exposed to successively increasing concentrations of substrate. The fastest growing strains are irradiated. The genetic alterations should increase the growth rate and fix the desired biochemical capability. Adaptation of the strains at high concentrations of a preferred substrate can induce increased production of the specific enzymes required to degrade that substrate (Thibault and Elliott, 1979). However, this is probably achieved at the expense of the ability to produce other enzymes. Therefore, an adapted mutant put back into the environment will only be able to compete against indigenous organisms when the preferred substrate is available.

Interstrain and interspecies genetic engineering promises to develop organisms with extraordinary abilities to degrade xenobiotic compounds (Chakrabarty, 1982). However, more information is needed on the ability of genetically engineered organisms to survive, grow, and function in the soil environment (Liang, Sinclair, Mallory, and Alexander, 1982). Other practitioners are skeptical because of the importance of the soil environment in determining the microbial activity and hence the success of applying exogenous organisms (Sims and Bass, 1984), especially since many compounds that can be degraded under laboratory conditions continue to persist in the environment (Pierce, 1982d).

An example of potential genetic engineering technology occurs with Pseudomonas putida (Hou, 1982; Jain and Sayler, 1987). Naturally occurring strains of the organism have the ability to degrade octanes (OCT plasmid), camphors (CAM plasmid), naphthalenes (NAH plasmid), toluene, and m- and p-xylenes, but no single strain is able to degrade all these pollutants simultaneously. By genetic and molecular techniques, it has been possible to transfer the plasmids NAH, TOL, and CAM-OCT to produce a multiplasmid Pseudomonas putida (MPP), thereby, constructing an organism that can transform, simultaneously, some linear alkanes and aromatic and polyaromatic hydrocarbons (Thibault and Elliott, 1979). This organism might be useful in the cleanup of oil spills or of wastes from industrial pollution. The creation of improved strains in this manner would accomplish the in situ treatment and removal of several environmental pollutants at the same time.

A number of genetically engineered strains have been developed. One successful application of the technique has been the development of a bacterial strain of Pseudomonas cepacia, capable of degrading 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Kellogg, Chatterjee, and Chakrabarty, 1981). After the compound was degraded in soil, the numbers dropped until more of the compound was added. The population increased until the material was degraded. No appreciable effects on the number or types of indigenous bacteria were noted. This organism still needs to be field tested.

A Flavobacterium sp. marine isolate degrades a variety of 2- and 3-ring PAHs (Foght and Westlake, 1985). It can use naphthalene or phenanthrene as sole carbon sources. Partial oxidation of anthracene and incomplete metabolism of dibenzothiophene occurs. This organism contains three large plasmids, which were initially refractory to isolation and demonstrated inconsistent stability in vitro. Complete curing was not achieved, but phenotypic variants, which showed plasmid involvement in PAH degradation, were generated.

Acinetobacter (P-7) can utilize a variety of growth substrates (Garvey, Stewart, and Yall, 1985). Growth was observed with n-alkanes (C_8 to C_{20} carbon chain length), camphor, phenethyl alcohol, and crude oil. This organism harbors a plasmid, pYGI. When cured of the plasmid, the ability to grow on decane, nonane, or octane was lost. Growth on larger carbon chain-length n-alkanes (C_{11} to C_{20}) was normal. Chromosomally mediated n-alkane utilization can be divided into three classes: C_{11} to C_{12}, C_{13} to C_{14}, and C_{15} to C_{20}.

Plasmids, such as TOL and NAH, mediate bacterial degradation of small aromatic compounds (Foght and Westlake, 1983). However, there is no evidence that plasmids play a role in degradation of larger, polycyclic aromatic hydrocarbons, such as phenanthrene, benzo(a)pyrene, or dibenzothiophene. Almost all aromatic degraders have at least one plasmid, which can be divided into two groups: those with limited ability to degrade aromatics (e.g., only biphenyls) have small plasmids; those with extensive capability to degrade PAHs have larger plasmids. Aromatic-degrading strains identified include members of the Flavobacterium, Acinetobacter, Pseudomonas, and Aeromonas genera. There is no evidence for any PAH-degrading isolates being capable of degrading saturates (e.g., n-alkanes) or vice versa.

Mutant bacterial formulations have been developed by Polybac Corporation to degrade complex organic materials, including industrial surfactants, crude and refined petroleum products, pesticides and herbicides, and solvents (see

Appendix C and Table C-3) (Thibault and Elliott, 1979). Polybac maintains an up-to-date library of information on the relative biodegradability of a wide range of organic chemicals by various mutant strains. This allows selection of the proper formulation of strains for a specific waste mixture to be treated.

Cytochrome P450 systems catalyze the monooxygenation of a broad range of xenobiotic compounds (Chen, Dey, Kalb, Sanglard, Sutter, Turi, and Loper, 1987). The individual P450 enzymes provide the specificity for a broad range of oxidative reactions. P450 systems are being engineered in yeasts for the oxidative detoxication and biodegradation of environmentally stable organic pollutants. Because of the highly substituted structure of the persistent polychlorinated aromatics, the P450 monooxygenases are likely candidates as catalysts for their specific oxidation or reductive dechlorination.

The hydroxylation of n-octane by Corynebacterium sp. 7EIC involves cytochrome P450 (Hou, 1982). Only n-alkanes with more than 14 carbon atoms are inducers of the synthesis of cytochrome P-450, the hydroxylase system, the NADPH-cytochrome c reductase, and cytochrome b.

Two organisms, Saccharomyces cerevisiae (baker's yeast) and Candida tropicalis ATCC750, a yeast capable of n-alkane assimilation, have been widely used in genetic studies (Chen, Dey, Kalb, Sanglard, Sutter, Turi, and Loper, 1987). S. cerevisiae is highly characterized genetically and has become the eukaryotic microorganism of choice for the application of molecular genetic techniques. C. tropicalis ATCC750 is representative of a group of yeasts capable of growth on n-alkanes in petroleum as a carbon and energy source. Such yeasts already express lipophilic properties that may be useful in genetically engineering cells for the uptake and metabolism of hydrophobic hazardous compounds. Access to the DNA sequence of the C.tropicalis gene for P450alk will facilitate testing of its expression as a foreign gene in S. cerevisiae. Experiments using these methods form important steps toward genetically engineering other specific P450s in yeasts for the degradation of hazardous wastes.

There are four major areas that may benefit from genetic engineering: stabilization; enhanced activity; multiple degradative activities; and health, safety, and environmental concerns (Pierce, 1982d).

1. Stabilization

The extrachromosomal-DNA (plasmids) responsible for degrading some hydrocarbons can be lost, if the organisms are not grown in the presence of these compounds (Pierce, Facklam, and Rice, 1980). As a result, the microbes lose the ability to degrade the material (Fisher, Appleton, and Pemberton, 1978). Selective pressure must be present to maintain these plasmid-encoded traits. Genetic engineering can stabilize these traits by constructing hybrid plasmids (Bassford, et al., 1980). This can involve insertion of desired genetic elements into a region where replication is under control of a conserved element.

2. Enhanced Activity

By taking a plasmid containing a degradative trait, which has a low number of copies of itself in a cell, and hybridizing it with another plasmid that has

a large number of itself present, more degrading enzyme may be produced (Pierce, 1982d). Optimization of the location of a promoter of protein systhesis will also increase the amount of protein produced (Shine and Dalgarno, 1975).

3. Multiple Degradative Activities

In nature, strains of Pseudomonas will contain only one degradative plasmid, since they belong to the same incompatibility group (Korfhagen, Sutton, and Jacoby, 1978). Incompatible plasmids are mutually exclusive, and an organism will contain only one from a given group. Fusion of the CAM, TOL, and OCT plasmids led to the construction of novel microorganisms with multiple degradative traits. Recombinant-DNA techniques could also be used to insert several elements into a single plasmid. Combinations can result in an organism with degradative traits different from either parent (Vandenbergh, Olsen, and Coloruotolo, 1981). Plasmid-assisted molecular breeding is also being used to produce novel degradative traits.

4. Health, Safety, and Environmental Concerns

The potential for adverse effects should be considered for wild-type and engineered strains (Pierce, 1982d). It is unlikely that recombinant-DNA techniques, as applied to microbial degradation, will result in additional biohazards. Strains may be constructed with a minimized potential for adverse effects, such as use of nontransmissible plasmids, reduction of the plasmid to the smallest size possible (removal of superfluous genetic information and cryptic genes), development of a host microorganism that will survive only under defined conditions, and construction of containment systems to limit the potential for escape.

Limiting Factors of Genetic Engineering

1. Compatibility and Coordination

Plasmid incompatibility must be overcome (Pierce, 1982d). The host must have the ability to synthesize the protein for the new degradative trait. A promoter must also be present to allow adequate genetic control of protein synthesis. The pathway or reactions encoded by the inserted plasmid must be compatible with the biochemical pathways encoded by the host chromosome. There is evidence that degradative plasmids are responsible for only the first several biochemical reactions of the degradative pathway, while the remainder is encoded by chromosomal genes (Fisher, Appleton, and Pemberton, 1978). There are also three pathways in pseudomonads for the degradation of aromatic compounds (ortho and meta cleavage and gentisate pathways), which further complicates the coordination of biochemical pathways and their regulation mechanisms (Doelle, 1975).

2. Environmental Conditions

Genetically engineered microorganisms may not be successful in the field (Pierce, 1982d). These microbes would have to compete with resident microflora. If there are already 10^8 to 10^9 bacteria/g soil, a very large number of the new organisms would have to be added for them to become established in the community. If environmental parameters, such as

temperature, pH, substrate concentration, oxygen tension, are not optimal for the added organisms, there will be reduced activity (e.g., a bacterium that degrades n-alkanes optimally at 30°C in an aqueous medium would probably perform poorly in an oil spill in the North Sea).

Microorganisms that are tolerant of multiple kinds of stresses (e.g., abiotic stress, starvation, and biological antagonism) have a higher potential for survival in the soil after genetic manipulation in the laboratory than organisms with less tolerance and versatility (Liang, Sinclair, Mallory, and Alexander, 1982). Genetic engineering is trying to produce bacteria capable of complete degradation of xenobiotic compounds by using zymogenous organisms, such as Pseudomonas (Chakrabarty, 1982). However, strains of Arthrobacter, more slowly growing autochthonous organisms, have also demonstrated xenobiotic degrading capabilities, and these have also shown potential for treating hazardous waste-contaminated soils (Stanlake and Finn, 1982; Edgehill and Finn, 1983).

There have been concerns that genetically engineered organisms may continue to persist in the environment (Stotsky and Krasovsky, 1981). It is hard to predict the fate of these organisms in such complex ecosystems as soils and aquatic environments. Unless the new genetic material confers a wider substrate range, or increases the organism's ability to detoxify its environment, it would seem energetically disadvantageous for the organism to maintain the genes. Eventually, the organism or its new genes would be selected against. Such an organism may decrease to unmeasureable numbers, if its target substrate is depleted (Chakrabarty, 1982). However, others argue that more stress-tolerant organisms could persist for extended periods (Stanlake and Finn, 1982; Edgehill and Finn, 1983).

Most of the bacteria typically used in microbial genetic work are eutrophs of the families Enterobacteriaceae and Pseudomonadaceae, which may not be able to attack substrates in the parts-per-billion range that are often found in environmental samples (Johnston and Robinson, 1982). Environmental stress, such as unsuitable water availability or the presence of toxicants may also affect an introduced microorganism. However, genetic engineering holds great promise, especially in treatment facilities where conditions can be controlled.

Since microbial growth doubles for about every 10°C increase in temperature, biodecontaminations would be slowed considerably in cool weather (Thibault and Elliott, 1979). Mutant organisms could be developed to provide the optimal degradation at any given temperature. Section 5.1.2.2 discusses the temperature dependency of the biodegradation of crude oil by a commercially available mutant bacterial formulation (PETROBAC® Mutant Bacterial Hydrocarbon Degrader). Biodegradation does occur at a temperature as low as 5°C.

It may not be feasible to engineer strains that are resistant to heavy metals, since it is more than likely that the toxic conditions found in industrial wastewaters will cause plasmid losses from organisms, even though they function well in the laboratory (Wood and Wang, 1983). It looks more promising to employ organisms, such as the green alga, Cyanidium caldarium, that naturally tolerate extreme conditions of pH and temperature.

Because of the large amount of information necessary before rational and reproducible experiments in strain construction can be performed, this approach

has not yet been applied on a wide scale in biodegradation research (Leisinger, 1983). Assembling degradative pathways from different bacterial strains in one cell by genetic manipulation is a laborious technique by which to obtain organisms with novel degradative capacities. This method will find its application in cases where a precise strategy can be formulated and where obstacles to degradation, as, for example, the intracellular accumulation of dead-end metabolites from problem compounds, cannot be overcome by cocultivation of strains with different degradative pathways.

The problem with the use of genetically selected microorganisms to degrade contaminants is that no conclusive evidence has been found that commercially available organisms are effective in establishing themselves or significantly enhancing biodegradation of pollutants in aeration basins or natural environments having an active native microbial population (Johnston and Robinson, 1982). In general, acclimated or genetically engineered organisms will not survive or offer significant advantage in treatment of hazardous wastes unless environmental parameters can be controlled to promote survival of the added organisms (Lee, Wilson, and Ward, 1987).

The addition of adapted mutant microbes has not been completely successful but has great potential (Knox, Canter, Kincannon, Stover, and Ward, 1968). A number of companies are involved in the production of microbial strains to be used to treat abandoned hazardous waste sites and chemical spills.

5.1.1.2 Use of Analog Enrichment for Cometabolism

Not all of the organic compounds at a contaminated site may be readily degraded (Atlas, 1981). It may be possible to induce oxidation of compounds that might otherwise not be degraded by addition of a second substrate that will promote cooxidation. Uncharacterized dissolved organic carbon may also play an important role in controlling the rate and extent of biodegradation of organic compounds present at low concentrations (Alexander, 1986).

Various organisms can be used in combination with chemical analogues of specific organic compounds to promote cooxidation of the latter. Some of these combinations, and the resultant metabolic products, are described below and summarized in Table A.5-2.

Sucrose has been used as a cometabolite in the degradation of 4,6-dinitro-o-cresol (DNOC), a phenolic priority pollutant, in wastewater, by use of an anaerobic recycle fluidized-bed reactor as a pretreatment stage, followed by an activated-sludge reactor as the aerobic treatment stage (Slonim, Lien, Eckenfelder, and Roth, 1985). There appeared to be a relationship between the sucrose concentration and degradation of the compound. A ratio of sucrose to DNOC of 2:1 or higher resulted in a 95 to 100 percent conversion of DNOC, while a lower ratio did not permit cometabolism or degradation of DNOC.

The rate of cometabolism of benzo(a)pyrene was significantly increased with enrichment of the soil with phenanthrene as an analog (Sims and Overcash, 1981). Similar results were obtained by using naphthalene, and especially phenanthrene, as growth substrates for the nongrowth substrates of pyrene, 3,4-benzpyrene, 1,2-benzanthracene, 1,3,5,6-dibenzanthracene, with approximately 35 percent of the nongrowth substrate remaining after four weeks (McKenna, 1977).

Table A.5-2. Chemical Analogues/Growth Substrates and Microorganisms for Cooxidation

Organic Compound	Analogue/ Growth Substrate	Organism	Products
Ethane, propane, butane[a]	Methane	*Pseudomonas methanica*	
2-Methylheptane[b]	Hexane	*Pseudomonas* sp.	Aldehydes, ketones, etc.
o-Xylene[b]	Hexane	*Pseudomonas* sp.	*o*-Toluic acid
Ethylcyclohexane[b]	Hexane	*Pseudomonas* sp.	Cyclohexane acid
o-Xylene[b]	Hexadecane	*Nocardia* sp.	*o*-Toluic acid
p-Xylene[b]	Hexadecane	*Nocardia* sp.	*p*-Toluic acid
Ethylcyclohexane[b]	Hexadecane	*Nocardia* sp.	Cyclohexane acid
Benzo(a)pyrene[c]	Phenanthrene		
Pyrene, 3,4-benzpyrene, 1,2-benzanthracene, 1,3,5,6-dibenzanthracene[d]	Phenanthrene, naphthalene		
4,6-Dinitro-o-cresol[e]	Sucrose		
Cycloparaffins[f]	Propane	*Mycobacterium vaccae*	Cycloalkanones
Cycloalkanes[f]	*n*-Heptane	*Pseudomonas aeruginosa*	Alcohols
n-Butylcyclohexane	*n*-Octadecane-grown *Nocardia* sp.		Cyclohexane acetic acid

a = (Foster, 1962)
b = (Jamison, Raymond, and Hudson, 1976)
c = (Sims and Overcash, 1981)
d = (McKenna, 1977)
e = (Slonim, Lien, Eckenfelder, and Roth, 1985)
f = (Hou, 1982)

It was discovered that Pseudomonas methanica cometabolically oxidized ethane, propane, and butane while the organism metabolized its growth substrate, methane (Foster, 1962). Soil aerobic organisms that can grow on aliphatic hydrocarbons, such as natural gas or propane, could possibly be added to the soil while methane or propane is pumped into the ground for degradation of a variety of chlorinated solvents, including TCE (Rich, Bluestone, and Cannon, 1986).

When two components of gasoline were combined, one for growth and one as a cooxidizable substrate, a Pseudomonas sp. was able to cooxidize 2-methylheptane, o-xylene, and ethylcyclohexane with hexane as the growth substrate (Jamison, Raymond, and Hudson, 1976). Nocardia sp. cooxidized primarily o-xylene and p-xylene. o-Xylene was oxidized to o-toluic acid, ethylcyclohexane to cyclohexane acid, p-xylene to p-toluic acid, and 2-methylheptane to a mixture of products, including ketones and aldehydes.

Bacteria capable of utilizing or cooxidizing phenanthrene were enumerated by spreading water and sediment samples on nutrient agar plates containing PHE as sole carbon source and plates containing PHE and one alternative carbon source (Shiaris and Cooney, 1981). Colonies producing on the first plates were classified as utilizers, and those producing clear zones only on the second were considered cooxidizers. The alternative substrates were effective in this order:

yeast extract/peptone > glucose > benzoic acid > oil/kerosene

Compounds with two or more chlorine atoms can be transformed anaerobically in the laboratory through reductive halogenation by addition of a primary substrate--acetate, methanol, or isopropanol--to increase the concentration and activity of methane-forming bacteria (Rich, Bluestone, and Cannon, 1986).

An alkane-utilizing strain of Mycobacterium vaccae (JOB5) cooxidizes a variety of alicyclic hydrocarbons to the corresponding ketones (Hou, 1982). A strain of Pseudomonas aeruginosa grown on n-heptane cooxidizes cycloalkanes to their corresponding alcohols. Cycloalkanes can be metabolized readily by mixed cultures of n-alkane utilizers, which cometabolize cycloalkanes, and other microorgnisms, which are able to utilize the alicyclic oxidation products. A Nocardia sp. produces biotin, which is utilized by a Pseudomonas strain to grow on cyclohexane.

Methane-grown microorganisms will stationary-oxidize alicyclic hydrocarbons. Stationary transformation is a term used to describe metabolism of nongrowth substrates by microorganisms in the absence of growth substrates. This is to be distinguished from cooxidation, in which the microorganisms require a growth substrate in order to metabolize the nongrowth compound.

The ability to use aromatic compounds can be an induced phenomenon in bacteria (Claus and Walker, 1964). Toluene-grown strains of Pseudomonas and Achromobacter oxidized, without lag, benzene, catechol, 3-methyl-catechol, benzyl alcohol, and, more slowly, o- and m-cresol, but not benzaldehyde or benzoic acid. The mutual adaptations to use benzene and toluene suggest that enzymes with similar activities may be involved in the metabolism of the two compounds. n-Octadecane grown Nocardia sp. was found to cooxidize n-butylcyclohexane to cyclohexane acetic acid (Hou, 1982).

Diauxie Effect

The opposite of cometabolism is a sparing, or diauxic, phenomenon, which occurs when a compound cannot be degraded in the presence of another compound (Atlas, 1981). The metabolic pathways of degradation are not altered, but the enzymes necessary for metabolic attack of a particular hydrocarbon may not be produced. This can lead to persistence of particular hydrocarbons in a petroleum mixture. Both processes of cooxidation and sparing can occur within the context of a petroleum spillage.

When a microorganism with a broad substrate range is offered more than one type of organic substrate, it will not attack the substrates simultaneously, but rather in a definite sequence (Bartha and Atlas, 1977). A diauxie effect, where the presence of one compound will inhibit the degradation of another, may determine whether or not the hydrocarbon components of an oil spill are degraded, and if so, in what order. A Brevibacterium erythrogenes strain was capable of utilizing pristane and other branched alkanes only in the absence of n-alkanes (Pirnik, Atlas, and Bartha, 1974). B. erythrogenes utilizes n-alkanes by a monoterminal beta-oxidation sequence, but degrades isoalkanes by diterminal oxidation. The common phenomenon that the n-alkane components of an oil spill disappear before the isoalkanes and other hydrocarbon classes show substantial biodegradative change strongly suggests that such diauxic regulatory mechanisms apply not only to some pure cultures, but most likely also to the mixed microbial community of the environment (Bartha and Atlas, 1977).

The presence of nonhydrocarbon substrates may repress the inductive synthesis of enzymes required for hydrocarbon oxidation (van Eyk and Bartels, 1968). Addition of glucose to lake water repressed hexadecane utilization by its microbial community in a diauxic manner (Bartha and Atlas, 1977). In another study, the rate of mineralization of organic compounds in trace concentrations was found to be enhanced by the addition or inorganic nutrients, arginine, or yeast extract, but reduced by addition of glucose (Rubin and Alexander, 1983). This effect should be considered when selecting a substrate as a cometabolite.

5.1.1.3 Application of Cell-free Enzymes

Enzymatic methods show promise for removing aromatic compounds from high strength industrial wastewater (Maloney, Manem, Mallevialle, and Fiessinger, 1985). Enzymatic oxidative coupling may be useful in eliminating aromatics that are not well removed in biological or physical water treatment. Wastewaters containing aromatic compounds are treated with horseradish peroxidase and hydrogen peroxide (Alberti and Klibanov, 1981). The resulting high molecular weight compounds are less soluble in water and can be removed by sedimentation or filtration.

One concern in the use of this technique for drinking water treatment is the nature of the products of the oxidative coupling (Maloney, Manem, Mallevialle, and Fiessinger, 1985). Biphenyls accounted for 3 percent of the initial carbon concentration (Schwartz and Hutchinson, 1981). They would be incomplete polymerization products and may be the predominant ones. They may not be removed by sedimentation or filtration and may pass through the treatment process in their altered (polymerized) form.

Another problem in drinking water treatment is the presence of competition or interfering compounds (Maloney, Manem, Mallevialle, and Fiessinger, 1985). Raw water supplies usually have background organic carbon composed mainly of humic acids (McCarty, 1980). It has been suggested that humic acids may deactivate peroxidase (Pflug, 1980).

The peroxidase-peroxide system is effective in eliminating chlorinated phenols from drinking water supplies, but does not remove their breakdown products from the water (Maloney, Manem, Mallevialle, and Fiessinger, 1985). Further work is necessary to determine if these by-products present a potential risk for human health and if they are removed in other unit processes. It is of interest that extracellular peroxidases have been found in soil (Kaufman, 1983). These could presumably be involved in a vast array of soil metabolic reactions affecting xenobiotic residues.

The Cetus Company developed a novel multienzyme process for the oxidation of propylene (Hou, 1982). The first enzyme reaction converts olefin to halohydrin in the presence of halide, hydrogen peroxide, and haloperoxidase. The latter can be obtained from horseradish, seaweed, or Caldariomyces. In the second reaction, propylene halohydrin is transformed to propylene oxide by halohydrin epoxidase or by whole cells of Flavobacterium sp.

Cell-free enzymes for treating hazardous waste constituents are not currently in bulk production (Munnecke, Johnson, Talbot, and Barik, 1982). Only eight companies accounted for 90 percent of worldwide production of industrial enzymes in 1981; five of the firms, in Western Europe. Only 16 enzymes (primarily amylases, proteases, oxidases, and isomerases) accounted for 99 percent of the 1981 market. This suggests that specialized enzyme production, even on a large scale, may be quite expensive. Current prices for bulk enzyme materials range in price from $1.45 to $164 per pound. If the enzyme can be produced through chemical synthesis, it will be much less expensive than if it is produced by microorganisms in fermenters.

5.1.1.4 Addition of Antibiotics

5.1.1.5 Effect of Biostimulation on Counts

The following accounts indicate the favorable influence biostimulation can have on the total counts or hydrocarbon-degrading organisms at actual field locations.

* After biostimulation at a site contaminated with gasoline, bacterial levels increased up to six million times the initial levels (Minugh, Patry, Keech, and Leek, 1983).

* From 10^2 to 10^5 gasoline-utilizing organisms/ml were found to be present in contaminated groundwater when preliminary tests were made before a bioreclamation operation began. The microbial population responded to the addition of nutrients and oxygen with a ten- to thousandfold increase in the numbers of gasoline-utilizing and total bacteria in the vicinity of the spill, with levels of hydrocarbon utilizers in excess of 10^6/ml in several wells. The microbial response was an order of magnitude greater in the sand than the groundwater.

* Aeration of the groundwater contaminated with methylene chloride, n-butyl alcohol, dimethyl aniline, and acetone (temperature 12 to 14°C) in a monitoring well with a small sparger and the subsequent addition of nutrients resulted in an increase of bacteria from 1.8×10^3/ml to 1.6×10^6/ml in a 7-day period (Jhaveri and Mazzacca, 1985).

* A natural flora of gasoline-utilizing organisms were present at levels of 10^3/ml (Jamison, Raymond, and Hudson, 1975) in an area contaminated with over 3,000 barrels of high-octane gasoline. This population could be increased a thousand-fold by supplementing the groundwater with air, inorganic nitrogen, and phosphate salts.

* At a contaminated site in Millville, New Jersey, a population of 10^2 to 10^5 gasoline-utilizing organisms/ml groundwater responded to the addition of nutrients and oxygen with a 10- to 1,000-fold increase in their numbers (Raymond, 1978). In several wells, levels of hydrocarbon-utilizers exceeded 10^6/ml.

* After biostimulation at a LaGrange, Oregon, site, bacterial levels increased up to 6 million times the initial levels (Minugh, Patry, Keech, and Leek, 1983).

* After the biostimulation program ended at Ambler, Pennsylvania, the numbers of gasoline-utilizing bacteria declined, suggesting a depletion of nutrients and gasoline (Raymond, Jamison, and Hudson, 1976).

* In the solvent contamination at the Biocraft Laboratories, Waldwick, NJ, the wells had populations of 10^3 to 10^4 colonies/ml prior to biostimulation; addition of nitrogen and phosphorus increased the numbers of resident organisms as high as four times that of the control level (Lee and Ward, 1985).

* At a site contaminated with over 3,000 barrels of high-octane gasoline, the natural flora of gasoline-utilizing organisms were present at levels of 10^3/ml (Jamison, Raymond, and Hudson, 1975). This population could be increased a thousandfold by supplementing the groundwater with air, inorganic nitrogen, and phosphate salts.

5.1.2 Optimization of Soil Factors

Biodegradation of contaminants in the soil can be enhanced by making environmental factors optimum for the required reactions. Table A.5-3 lists the site and soil properties that should be identified to predict potential migration of a contaminant and to indicate the factors that will have to be adjusted to achieve optimum biodegradation (JRB Associates, Inc., 1984b).

The inherent capacity of soil to degrade toxicants by chemical and biological mechanisms can be maximized by identification of the soil conditions that promote the degradation of each toxicant and manipulation of the soil environment to bring about these conditions (Arthur D. Little, Inc., 1976). Although each toxicant, in general, has a unique set of ideal soil conditions for degradation, for some compounds these ideal conditions overlap, and more than one toxic substance can be the focus of soil manipulation at one time.

Table A.5-3. Important Site and Soil Characteristics for In Situ Treatment (JRB Associates, Inc., 1984b)

Site location/topography and slope
Soil type, and extent
Soil profile properties
Boundary characteristics
Depth
** Texture
Amount and type of coarse fragments
** Structure
Color
Degree of mottling
** Bulk density
Clay content
Type of clay
** Cation exchange capacity
* Organic matter content
* pH
* Eh
* Aeration status
Hydraulic properties and conditions
Soil water characteristic curve
Field capacity/permanent wilting point
** Water holding capacity
** Permeability (under saturated and a range of unsaturated conditions)
* Infiltration rates
Depth to impermeable layer or bedrock
* Depth to groundwater, including seasonal variations
Flooding frequency
* Run-off potential
Geological and hydrogeological factors
Subsurface geological features
* Groundwater flow patterns and characteristics
Meteorological and climatological data
Wind velocity and direction
Temperature
Precipitation
Water budget

* Factors that may be managed to enhance soil treatment
** Factors that may be managed to enhance soil treatment with shallow depth contamination.

For other compounds, the ideal conditions do not overlap and are sometimes even contradictory; these materials must be treated in series.

Table A.5-4 lists the soil factors that may have to be modified during the use of various treatment technologies (Sims and Bass, 1984). The most important soil factors that affect biodegradation are water content, temperature, soil pH, oxygen supply, available nutrients, oxidation-reduction potential, and soil texture and structure (Hornick, 1983).

5.1.2.1 Soil Moisture

Bacterial activity is highest in the presence of moisture (JRB Associates, Inc., 1984b). The greatest diversity and activity of microorganisms and the highest population densities are consistently observed in the sandy water-bearing strata, whereas, the dense, dry-clay layer zones have the least microbiological activity (Fredrickson and Hicks, 1987). The aerobic biodegradation of simple or complex organic material in soil is commonly greatest at 50 to 70 percent of the soil water-holding (field) capacity (Pramer and Bartha, 1972; Zitrides, 1983). Inhibition at lower values is due to inadequate water activity, and higher values interfere with soil aeration. Excess moisture, extremely dry conditions, pooling, or flooding should be avoided (Zitrides, 1983). Biodecontamination programs should not be conducted during heavy rains or drought. However, an observed lack of inhibition at 30 percent of the field capacity suggests that the moisture requirement for maximum activity on hydrophobic petroleum may be different than the optimal moisture levels for the biodegradation of hydrophilic substrates (Dibble and Bartha, 1979a).

Rainfall is useful to biodegradation. It dissolves contaminants and acts as a carrier as it percolates through the soil on its way to the groundwater (Dietz, 1980). Rainwater also keeps the contaminated soil moist, and microorganisms will utilize the oxygen dissolved in interstitial water droplets (Thibault and Elliot, 1980).

Many organisms are capable of metabolic activity at water potentials lower than -15 bar (Soil Science Society of America, 1981). The lower limit for all bacterial activity is probably about -80 bar, but some organisms cease activities at -5 bar. Although many microbial functions continue in soils at -15 bars or drier, optimum biochemical activity is usually observed at soil water potentials of -0.1 to 1.0 bar (Sommers, Gilmore, Wildung, and Beck, 1981). The kinds of microorganisms that are metabolically active in the soil will be affected. Degradation rates are highest at soil water potential between 0 and -1 bar. When natural precipitation cannot maintain near optimal soil moisture for microbial activity, irrigation may be necessary (Sims and Bass, 1984). Although degradation of hazardous organic compounds may be accelerated by soil moisture optimization, more rapid treatment of the contaminated soil may be achieved when moisture augmentation is used in combination with other techniques.

Moisture control is widely practiced in agriculture; however, there is little information available on its use to stimulate biological degradation of hazardous materials in soil (Sims and Bass, 1984). Most laboratory studies have been conducted at or near optimal soil moisture. The success of this technology depends upon the biodegradability of the waste constituents and the

Table A.5-4. Soil Modification Requirements for Treatment Technologies (Sims and Bass, 1984)

Technology	Oxygen Content	Moisture Content	Nutrient Content	pH	Temperature
EXTRACTION	-	-	-	x	x
IMMOBILIZATION					
Sorption (heavy metals)					
Agri. products	-	-	-	x	-
Activated carbon	-	-	-	x	-
Tetren	-	-	-	x	-
Sorption (organics)					
Soil moisture	-	x	-	-	-
Agri. Products	-	-	-	-	-
Activated carbon	-	-	-	-	-
Ion exchange					
Clay	-	-	-	x	-
Synthetic resins	-	-	-	x	-
Zeolites	-	-	-	x	-
Precipitation					
Sulfides	x	x	-	x	-
Carbonates, phosphates, and hydroxides	x	x	-	x	-
DEGRADATION					
Oxidation					
Soil-catalyzed reactions	x	-	-	x	-
Oxidizing agents	x	-	-	x	-
Reduction					
Reducing agents	x	x	-	x	-
Chromium	x	-	-	x	-
Selenium	x	-	-	x	-
PCBs and Dioxins	-	x	-	-	x
Polymerization	-	-	-	-	-
Modification of soil properties (for biodegradation)					
Soil moisture	-	x	-	-	-
Soil oxygen--aerobic	x	-	-	-	-
Soil oxygen--anaerobic	x	x	-	-	-
Soil pH	-	-	-	x	-
Nutrients	-	-	x	-	-

Table A.5-4. Soil Modification Requirements for Treatment Technologies (Sims and Bass, 1984) (Continued)

Technology	Oxygen Content	Moisture Content	Nutrient Content	pH	Temperature
Nonspecific org. amendments	-	-	-	-	x
Analog enrichment for cometabolism	-	-	-	-	x
Exogenous acclimated or mutant microorganisms	-	-	x	-	x
Cell-free enzymes	-	-	-	-	x
Photolysis					
Proton donors	-	-	-	-	-
Enhance volatilization	-	x	-	-	-
ATTENUATION					
Metals	-	-	-	-	-
Organics	-	-	-	-	-
REDUCTION OF VOLATILES					
Soil Vapor Volume	-	x	-	-	-
Soil Cooling	-	-	-	-	x

x = required

- = not required

suitability of the site and soil for moisture control. Its effectiveness may be enhanced by combination with other treatment techniques to increase biological activity. The technology is reliable in that it has been used in agriculture, but retreatment is necessary. Leaching of soluble hazardous compounds may occur, and erosion may also be a problem.

Control of soil moisture content can be practiced to optimize degradative and sorptive processes and may be achieved by several means (Sims and Bass, 1984). Supplemental water may be added to the site (irrigation), excess water may be removed (drainage, well points), or these methods can be combined with other techniques, such as using soil additives, for greater moisture control.

5.1.2.1.1 Irrigation

Soil may be irrigated by subirrigation, surface irrigation, or overhead (sprinkler) irrigation (Fry and Grey, 1971). With subirrigation, water is applied below the ground surface and moves upward by capillary action. If the water has high salinity, salts may accumulate in the surface soil, with an adverse effect on soil microbiological activity. The site must be nearly level and smooth, with either a natural or perched water table, which can be maintained at a desired elevation. The groundwater is regulated by check dams and gates in open ditches, or jointed perforated pipe to maintain the water level in the soil. Use of such systems is limited by the restrictive site criteria. There may be situations in which a subirrigation system may be combined with a drainage system to optimize soil moisture content. However, at a hazardous waste site, raising the water table might result in undesirable groundwater contamination.

With trickle irrigation, filtered water is supplied directly on or below the soil surface through an extensive pipe network with low flow-rate outlets only to areas that require irrigation. It does not give uniform coverage to an area, but with proper management, does reduce percolation and evaporation losses. For most in-place treatment sites, this method would probably not be appropriate, but it may be applicable in an area where only "hot spots" of wastes are being treated.

Surface irrigation includes flood, furrow, or corrugation irrigation. Since the prevention of off-site migration of hazardous constituents to ground- or surface waters is a primary restraint on a treatment technology, surface irrigation should be considered with caution. Contaminated water may also present a hazard to on-site personnel.

In flood irrigation, water covers the surface of a soil in a continuous sheet. Theoretically, water should stay at every point just long enough to apply the desired amount, but this is difficult or impossible to achieve under field conditions.

In furrow irrigation, water is applied in narrow channels or furrows. As the water runs down the furrow, part of it infiltrates the soil. Considerable lateral movement of the water is required to irrigate the soil between furrows. Salts also tend to accumulate in the area between furrows. Furrow irrigation frequently requires extensive land preparation, which usually would not be possible or desirable at a hazardous waste site due to contamination and safety considerations.

In corrugation irrigation, as with furrow irrigation, water is applied in small furrows from a head ditch. However, in this case, the furrows are used only to guide the water, and overflooding of the furrows can occur.

In general, control and uniform application of water is difficult with surface irrigation. Also, soils high in clay content tend to seal when water floods the surface, limiting water infiltration.

The basic sprinkler irrigation system consists of a pump to move water from the source to the site, a pipe or pipes leading from the pump to the sprinkler heads, and the spray nozzles. Sprinkler irrigation has many advantages. Erosion and run-off of irrigation water can be controlled or eliminated, application rates can be adjusted for soils of different textures, even within the same area, and water can be distributed more uniformly. Sprinkler irrigation is also possible on steep, sloping land and irregular terrain. Usually less water is required than with surface flooding methods, and the amount of water applied can be controlled to meet the needs of the in-place treatment technique. Also, a larger soil surface area is covered, which could facilitate soil washing.

There are several types of sprinkler irrigation systems:

1. Permanent installations with buried main and lateral lines

2. Semipermanent systems with fixed main lines and portable laterals

3. Fully portable systems with portable main lines and laterals, as well as a portable pumping plant

The first two types (especially the first) would likely not be appropriate nor cost-effective for a hazardous waste site because of the required land disturbance for installation and the limited time period for execution of the treatment.

The fully portable systems may have hand-moved or mechanically moved laterals. Portable systems can be installed in difficult areas, such as forests, in a way that will avoid interfering with trees. Mechanically moved laterals may be side-roll/wheel-move, center-pivot systems, or traveling sprinklers. This equipment is more expensive, but it requires considerably less labor than hand-moved systems. The health and safety of the workers must be considered, as well as the cost in the choice of an appropriate system.

5.1.2.1.2 Drainage

When irrigation is used, controls for erosion and proper drainage due to run-off are necessary (Sims and Bass, 1984). A properly designed drainage system removes excess water or lowers the groundwater level to prevent waterlogging (Fry and Grey, 1971). Open ditches and lateral drains are used for surface drainage, while a system of open ditches and buried tube drains into which water seeps by gravity is used for subsurface drainage. The collected water is conveyed to a suitable disposal point. Subsurface drainage may also be accomplished by pumping from wells to lower the water table. The drainage water to be disposed of off-site must not be contaminated with

hazardous substances, and must be collected, stored, treated, or recycled if not acceptable for off-site release.

Surface drains are used where subsurface drainage is impractical (e.g., impermeable soils, excavation difficult) to remove surface water or lower the water table (Donnan and Schwab, 1974). Subsurface drains are used to lower the water table. Construction materials include clay or concrete tile, corrugated metal pipe, and plastic tubing. Selection depends upon strength requirements, chemical compatibility, and cost.

5.1.2.1.3 Well Points

Like subsurface drains, well points can be used to lower the water table in shallow aquifers. They typically consist of a series of riser pipes screened at the bottom and connected to a common header pipe and centrifugal pump. Well point systems are practical up to 10 m (33 ft) and are most effective at 4.5 m (15 ft). However, their effectiveness depends upon site-specific conditions, such as the horizontal and vertical hydraulic conductivity of the aquifer (Ehrenfeld and Bass, 1983).

5.1.2.1.4 Additives

Various additives are available to enhance moisture control; e.g., the water-retaining capacity of the soil can be enhanced by adding water-storing substances (Nimah, Ryan, and Chaudhry, 1983). Water-repelling agents are available to diminish water absorption by soils. Water-repelling soils can be treated with surface-active wetting agents to improve water infiltration and percolation. Surface-active agents also accelerate soil drainage, modify soil structure, disperse clays, and make soil more compactable. There are also evaporation retardants to retain moisture in a soil.

5.1.2.2 Soil Temperature

A temperature gradient exists in the soil (Ahlert and Kosson, 1983). As a result of heat transfer phenomena, temperature responds less to daily weather fluctuations at increased depths. Microorganisms near the surface of the soil column must adapt more readily to temperature fluctuations than those at greater depth.

Soil temperature can be modified by soil moisture control and by the use of mulches of natural or artificial materials (JRB Associates, Inc., 1984b). Mulches can affect soil temperature in several ways. In general, they reduce diurnal and seasonal fluctuations in soil temperature (Sims and Bass, 1984). In the middle of summer, there is little overall temperature difference between mulched and bare plots, but mulched soil is warmer in spring, winter, and fall, and warms up more slowly in the spring.

Mulches with low thermal conductivities decrease heat flow both into and out of the soil; thus, soil will be cooler during the day and warmer during the night. White paper, plastic, or other types of white mulch increases the reflection of incoming radiation; thereby reducing excessive heating during the day. A transparent plastic mulch transmits solar energy to the soil and produces a greenhouse effect. A black paper or plastic mulch absorbs radiant energy during the day and reduces heat loss at night. Placing a black covering

over the soil to increase the soil temperature during the winter has been suggested as a means of overcoming the problem of slower biodegradation at the lower winter temperatures (Guidin and Syratt, 1975). Humic substances also increase soil temperature by their dark color, which increases the surface soil's heat absorption (Sims and Bass, 1984). Use of film mulch as a means of stimulating waste oil biodegradation by increasing soil temperatures during the winter, however, would preclude tilling of the soil and, thus, decrease its aeration (Dibble and Bartha, 1979a). Some workers believe this would not have an overall beneficial effect and may, in fact, be unnecessary, since it has been reported that the albedo decrease due to oil contamination raised the temperature in the upper 10 to 20 cm of tundra soils by as much as $5^{o}C$ (Freedman and Hutchinson, 1976).

The type of mulch required determines the application method (Sims and Bass, 1984). Mulches are also used to protect soil surfaces from erosion, reduce water and sediment run-off, prevent surface compaction or crusting, conserve moisture, and help establish plant cover (Soil Conservation Service, 1979). Commercial machines for spraying mulches are available. Hydromulching is a process in which seed, fertilizer, and mulch are applied as a slurry. To apply plastic mulches, equipment that is towed behind a tractor mechanically applies plastic strips that are sealed at the edges with soil. For treatment of large areas, special machines that glue polyethylene strips together are available (Mulder, 1979). Table C-9 describes the organic materials available for use as mulch and the situations when each would be most suitable.

Irrigation increases the heat capacity of the soil, raises the humidity of the air, lowers air temperature over the soil, and increases thermal conductivity, resulting in a reduction of daily soil temperature variations (Baver, Gardner, and Gardner, 1972). Sprinkle irrigation, for example, has been used for temperature control, specifically frost protection in winter and cooling in summer and for reduction of soil erosion by wind (Schwab, Frevert, Edminster, and Barhes, 1981). Drainage decreases the heat capacity, thus, raising the soil temperature. Elimination of excess water in spring causes a more rapid temperature increase. The addition of humic substances improves soil structure, thus improving soil drainability, resulting indirectly in increasing the insulative capacity of the soil.

Several physical characteristics of the soil surface can be modified to alter soil temperature (Baver, Gardner, and Gardner, 1972). Compaction of the soil surface increases the density and, thus, the thermal conductivity. Tillage, on the other hand, creates a surface mulch that, when dry, reduces heat flow from the surface to the subsurface. The diurnal temperature variation in a cultivated soil is often much greater than in an untilled soil. A loosened soil has more surface area exposed to the sun but is colder at night and more susceptible to frost.

Raising the temperature of a contaminated zone can also be achieved by pumping in heated water or recirculating groundwater through a surface heating unit (Environmental Protection Agency, 1985b).

Since microbial growth doubles for about every $10^{o}C$ increase in temperature, biodecontaminations can be slowed considerably in cool weather (Thibault and Elliott, 1979). Mutant organisms are being developed to provide the optimal degradation at any given temperature. Figure A.5-1 shows the

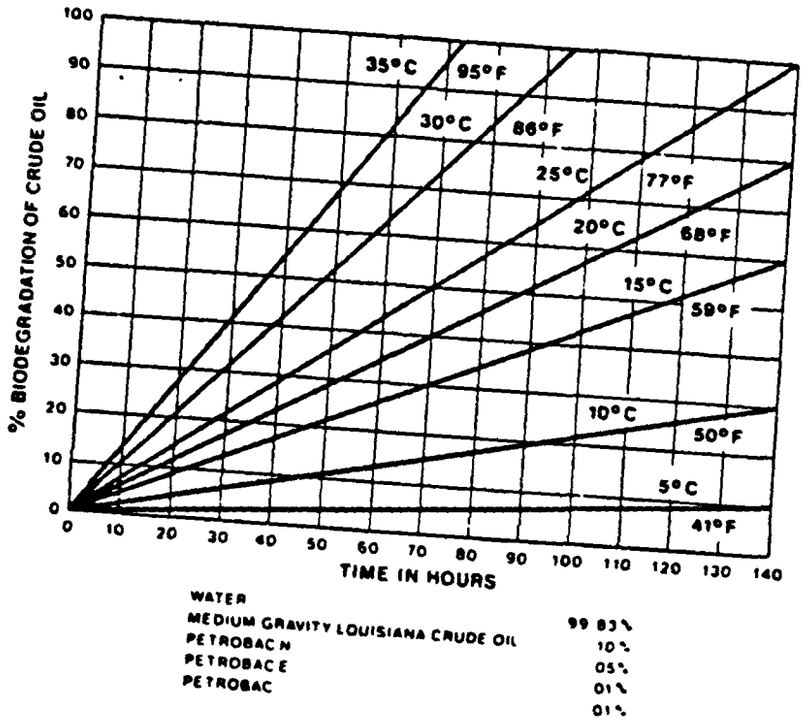


Figure A.5-1. Temperature Dependency of Biodegradation (Thibault and Elliott, 1979)

temperature dependency of the biodegradation of crude oil by a commercially available mutant bacterial formulation (PETROBAC® Mutant Bacterial Hydrocarbon Degrader) (Thibault and Elliott, 1979).

5.1.2.3 Soil pH

5.1.2.3.1 Increasing Soil pH

Liming is a frequent agricultural practice and is the most common method of controlling pH, while acidification is much less common (Sims and Bass, 1984). Methods have been developed to determine the lime requirement of soils, taking into account the buffering capacity of the soil (McLean, 1982). A lime requirement test may be performed to find the loading rate to use for increasing soil pH. However, there are no readily available guidelines for reducing soil pH, and the acidification requirements for a particular soil have to be determined experimentally in the laboratory, taking into account the buffering capacity of the waste. Thorough mixing is required in the zone of contamination to change the pH. Run-off and minor controls are necessary to control drainages and erosion of the tilled soil. The achievable level of treatment is high, depending upon the wastes, site, and soil. It may be necessary to repeat the process during the treatment.

Liming is the addition to the soil of any calcium or calcium- and magnesium-containing compound capable of reducing acidity (i.e., raising pH) (Sims and Bass, 1984). Lime correctly refers only to calcium oxide, but is commonly used to refer to calcium hydroxide, calcium carbonate, calcium-magnesium carbonate, and calcium silicate slags.

There are several benefits of liming to biological activity (Sims and Bass, 1984). At higher pH values, aluminum and manganese are less soluble; both of these compounds are toxic to most plants. In addition, phosphates and most microelements necessary for plant growth (except molybdenum) are more available at higher pH. Microbial activity is greater at or near neutral pH, which enhances mineralization and degradation processes and nitrogen transformations (e.g., nitrogen fixation and nitrification).

The liming material to use depends upon several factors (Sims and Bass, 1984). Calcitic and dolomitic limestones are the most commonly used materials. However, these must be ground in order to be effective quickly, since the velocity of reaction is dependent upon the surface in contact with the soil. The finer they are ground, the more rapidly they react with the soil. A more finely ground product usually contains a mixture of fine and coarse particles to effect a rapid pH change and still be relatively long-lasting, as well as reasonably priced. Many states require that 75 to 100 percent of the limestone pass an 8- to 10-mesh sieve and that 20 to 80 percent pass anywhere from an 8- to 100-mesh sieve. Calcium oxide and calcium hydroxide are manufactured as powders and react quickly. Other factors to consider in the selection of a limestone are neutralizing value, magnesium content, and cost per ton applied to the land.

Lime requirement for soil pH adjustment depends on soil factors, such as soil texture, type of clay, organic matter content, and exchangeable aluminum (Follett, Murphy, and Donahue, 1981). The buffering capacity reflects the soil cation exchange capacity and will directly affect the amount of lime required

to adjust soil pH. The amount of lime required is also a function of the depth of incorporation at the site; i.e., volume of soil to be treated. The amount of lime necessary to effect a pH change in a particular site/soil/waste system is determined by a commercial soil testing laboratory in short-term treatability studies or soil-buffer tests (McLean, 1982). Lime requirements are also affected by acid-forming fertilizers. Commonly used liming materials are summarized in Table C-10.

Limestone does not migrate easily in the soil since it is only slightly soluble and must be placed where needed (Sims and Bass, 1984). Plowing or discing surface-applied lime into the soil may, therefore, be required. The application of fluid lime is becoming more popular, especially when mixed with fluid nitrogen fertilizer. This combination results in fewer passes over the soil, and the lime is available to counteract acidity produced by the nitrogen. Also, limestone has been applied successfully to a pharmaceutical wastewater land treatment facility through a spray irrigation system.

The addition of basic waste to acidic soil increases the pH of the surface layer (4" to 18") but not the subsoil (Brown, 1975). The reaction neutralizes the buffer capacity of the soil. Basic waste can cause physical damage to the soil system; however, weak organic bases added to the soil may increase the soil buffer capacity and exchange capacity as the bases are degraded.

5.1.2.3.2 Decreasing Soil pH

Ferrous sulfate can be added to the soil to decrease alkalinity (Arthur D. Little, Inc., 1976). Under acidic conditions in soils, solubilities of complexed cations, such as copper (Cu) and zinc (Zn), increase, and iron (Fe), manganese (Mn), and Cu are easily reduced to more soluble forms (JRB Associates, Inc., 1984b). Acidic wastes may also be used as a treatment process for saline-sodic soils.

5.1.2.4 Oxygen Supply

Biodegradation of most organic contaminants requires approximately two parts of oxygen to completely metabolize one part of organic compound (Wilson, Leach, Henson, and Jones, 1986). The complete oxidation of 1 mg of hydrocarbon to carbon dioxide and water requires 3 to 4 mg of oxygen (Texas Research Institute, Inc., 1982). Less oxygen is needed when microbial biomass is generated or when oxidation is not complete. Typically, about half of the carbon in hydrocarbons is converted to biomass (Green, Lee, and Jones, 1981). The problem is providing the necessary amount to the site where it will be used. Oxygen can be provided to the subsurface through the use of air, pure oxygen, hydrogen peroxide, or ozone (Environmental Protection Agency, 1985b). Fluid and semisolid systems can be aerated by means of pumps, propellers, stirrers, spargers, sprayers, and cascades (Texas Research Institute, Inc., 1982). The advantages and disadvantages of various oxygen supply alternatives are summarized in Table A.5-5 (Environmental Protection Agency, 1985b).

A great deal of water would be needed in fine-textured subsurface materials to supply the necessary oxygen to promote biodegradation (Wilson, Leach, Henson, and Jones, 1986). Oxygen levels can be increased about fivefold by sparging injection wells with oxygen instead of air. Laboratory studies have shown that hydrocarbon-degrading bacteria can adapt to tolerate hydrogen

Table A.5-5. Oxygen Supply Alternatives (Environmental Protection Agency, 1985b)

Substance	Application Method	Advantages	Disadvantages
Air	In-line	Most economical	Not practical except for trace contamination <10 mg/l COD
	In situ wells	Constant supply of oxygen possible	Wells subject to blow out
Oxygen-enriched air or pure oxygen	In-line	Provides much higher oxygen solubility than air	Not practical except for low levels of contamination <25 mg/l COD
	In situ wells	Constant supply of oxygen possible	Very expensive, wells subject to blow out
Hydrogen peroxide	In-line	Moderate cost, intimate mixing with groundwater, greater oxygen concentrations can be supplied to subsurface (100 mg/l), H_2O_2 provides 50 mg/l oxygen, helps to keep wells free of heavy growth	Chemical decomposes rapidly on contact with soil, and oxygen may bubble out prematurely unless properly stabilized
Ozone	In-line	Chemical oxidation will occur, rendering compounds more biodegradable	Ozone generation is expensive, toxic to microorganisms except at low concentrations, may require additional aeration

peroxide equivalent to 200 mg/l oxygen, a twentyfold increase in oxygen over water sparged with air (Lee and Ward, 1987). However, the rate of decomposition of hydrogen peroxide to oxygen must be controlled. Rapid decomposition of only 100 mg/l hydrogen peroxide will exceed the solubility of oxygen in water, resulting in bubble formation, which could lead to gas blockage and loss of permeability.

The flow of oxygen into the system is controlled by oxygen concentration in the carrier and the permeability of the geological material to that carrier (Wilson, Leach, Henson, and Jones, 1986). Air is much less viscous than water (1.8×10^2 and 1.0×10^4 upoise, respectively). Air also has a twentyfold greater oxygen content on a volume basis. If the air- and water-filled porosity are about the same, and the pressure gradients are the same, then air should be about 1000 times more effective than water. Air should be particularly effective for oxygen supply to contaminated regions high in the unsaturated zone.

Whether the contaminant is above or below the water table, the rate of bioreclamation in hydrocarbon-contaminated zones is effectively controlled by the rate of supply of oxygen (Wilson, Leach, Henson, and Jones, 1986). Table A.5-6 compares the number of times that water in contaminated material below the water table, or air in material above it, must be replaced to totally reclaim subsurface materials of various textures. The calculations assume typical values for the volume occupied by air, water, and hydrocarbons (De Pastrovich, Baradat, Barthal, Chiarelli, and Fussel, 1979). The actual values at a specific site will probably be different. It is also assumed that the oxygen content of the water is 10 mg/l, that of the air 200 mg/l, and that the hydrocarbons are completely metabolized to carbon dioxide.

After the oxygen in the air is consumed during the biological degradation of the contaminant, the remaining air should physically weather (remove volatiles by evaporation) the hydrocarbons (Wilson, Leach, Henson, and Jones, 1986). The extent of weathering depends upon the vapor pressure of the contaminant. Light hydrocarbons, such as gasoline, can be vaporized to a greater extent than they are metabolized with oxygen. The vapor pressure of gasolines varies from 100 to 1000 mm at 100°C. If the vapor pressure is reduced fourfold at typical groundwater temperatures of 10°C, and benzene is typical of the vapors, then the oxygen demand for complete metabolism of the gasoline vapors ranges from twice to 20 times the oxygen content of air. The biological and physical weathering of the hydrocarbon should preferentially remove the more volatile and more water-soluble components, which are the greatest hazards to groundwater quality (De Pastrovich, Baradat, Barthal, Chiarelli, and Fussel, 1979).

There is another treatment approach worth considering. If preliminary remediation has removed any hydrocarbons floating on the water table, and if the geology is favorable, then it might be possible to lower the water table to bring the entire contaminated soil into the unsaturated zone where it is available to permeation by air (Wilson, Leach, Henson, and Jones, 1986).

Table A.5-6. Estimated Volumes of Water or Air Required to Completely Renovate Subsurface Material that Originally Contained Hydrocarbons at Residual Saturation (Atlas, 1978c)

Texture	Proportion of the total volume of the subsurface occupied by			Volumes required to meet the oxygen demand of the hydrocarbons	
	Hydrocarbons when drained	Air when drained	Water when flooded	Air	Water
Stone to coarse gravel	0.005	0.4	0.4	250	5000
Gravel to coarse sand	0.008	0.3	0.4	530	8000
Coarse to medium sand	0.015	0.2	0.4	1500	15,000
Medium to fine sand	0.025	0.2	0.4	2500	25,000
Fine sand to silt	0.040	0.2	0.5	4000	32,000

There are a multitude of chemical, photosynthetic, and electrochemical reactions that produce oxygen, either as a major or minor product (Texas Research Institute, Inc., 1982). The chemical reaction types most often encountered are:

1. Decomposition of peroxides, superoxides (Shanley and Edwards)

H_2O_2 --------► $H_2O + 1/2O_2$ (a good, ecologically sound additive, used extensively in sewage treatment
Hydrogen peroxide

$Na_2O_2 + H_2O$ --------► $NaOH + H_2O_2$
Sodium peroxide

$NaO_2 + H_2O$ --------► $Na_2O_2 + O_2$
Sodium superoxide

Barium and strontium peroxides are used in the production of oxygenating cakes employed by fishermen for maintaining live bait (Texas Research Institute, Inc., 1982). A typical formulation would contain barium peroxide, manganese dioxide, calcium sulfate, and dental plaster, which releases oxygen slowly when in contact with water. However, use of materials such as these may not be advisable, because of the resulting heavy metal contamination of the water table. Barium peroxide is definitely highly poisonous.

There is also a urea-peroxide addition compound that has been used in conjunction with phosphate solutions to treat plants suffering from oxygen starvation in the root zone (U.S. Patent 3,912,490) (Texas Research Institute, Inc., 1982). The compound is available commercially from Western Europe. It is probably of the inclusion type, one in which H_2O_2 molecules are trapped within channels formed by the crystallization of urea (March, 1968). Since the molecules are held together only by Van der Waal's forces, when dissolved, the solution will behave as a mixture of urea and hydrogen peroxide. By weight, 35 percent of the compound is H_2O_2.

2. Decomposition of peroxyacids and salts (Austin Amer. Statesman, 1980)

Peroxy mono- and disulfuric acids, peroxy mono- and diphosphoric acid, and peroxyborates all produce acidic solutions, but the salts may be important for consideration.

The exact mode of degradation of the salt, $KHSO_5$ (potassium monoperoxy sulfate), is uncertain, but it has been used as an aid in the degradation of atrazine (a pesticide)--presumably by virtue of its oxygen-producing ability. Degradation probably results in the formation of H_2O_2 and $KHSO_4$.

Impure salts of peroxy monophosphoric acid (H_3PO_5) might prove useful.

3. Thermal decomposition of oxygen-bearing salts

$2NaNO_3$ --------► $2NaNO_2 + O_2$
heat

$2KClO_3$ --------► $2KCl + 3O_2$
heat

Generation of oxygen by this method has no particular advantage to treating underground contamination.

Two powerful oxidizing agents that do have potential for in-place treatment are ozone and hydrogen peroxide.

5.1.2.4.1 Ozone

Ozone is an oxygen molecule containing three oxygen atoms (Roberts, Koff, and Karr, 1988). Ozone gas is a very strong oxidizing agent that is very unstable and extremely reactive (Environmental Protection Agency, 1985b). It cannot be shipped or stored; therefore, it must be generated on-site prior to application.

Ozone may be used to degrade recalcitrant compounds directly by creating an oxygenated compound without chemical degradation (Texas Research Institute, Inc., 1982). Ozonation is an oxidation process appropriate for aqueous streams that contain less than 1.0 percent oxidizable compounds (Roberts, Koff, and Karr, 1988). This chemical oxidation can be used on many organic compounds that cannot be easily broken down biologically, including chlorinated hydrocarbons, alcohols, chlorinated aromatics, pesticides, and cyanides (Lee and Ward, 1986).

Ozone can also be used to increase the dissolved oxygen level in the water for enhancing biological activity (Texas Research Institute, Inc., 1982). It can be employed as a pretreatment for wastes to break down refractory organics or to furnish a polishing step after biological or other treatment processes to oxidize untreated organics (Roberts, Koff, and Karr, 1988). The rate of ozone reaction can be controlled by adjusting the pH of the medium (Texas Research Institute, Inc., 1982). At high pH, hydroxyl free-radical reactions dominate over the more rapid direct ozone reactions.

The most effective and cost-effective uses of ozone in soil system decontamination appear to be in the treatment of contaminated water extracted from contaminated soil systems through recovery wells, and in the stimulation of biological activity in saturated soil (Nagel, et al., 1982). Ozone treatment may be very effective for enhancing biological activity, if the organic contaminants are relatively biodegradable. However, if much of the material is relatively biorefractory, the amount of ozone required would greatly increase the cost of the treatment.

Ozone has been used to treat groundwater contaminated with oil products to reduce dissolved organic carbon concentration (Nagel, et al., 1982). Dosages of 1 g ozone/g dissolved organic carbon resulted in residual water ozone concentration of 0.1 to 0.2 ppm. The treated water was then infiltrated into the aquifer through injection wells. There was an increase in dissolved oxygen in the contaminated water. This increased microbial activity in the saturated soil zone, which stimulated microbial degradation of the organic contaminants.

Ozone was used to treat a petroleum contamination in Karlsruhe, Germany, that threatened a drinking water supply (Atlas and Bartha, 1973e). The polluted groundwater was withdrawn, treated with ozone, and infiltrated back into the system *via* three infiltration wells. About 1 g ozone/g DOC was added to the groundwater, which increased the biodegradability of the petroleum

contaminants and added dissolved oxygen. The dissolved oxygen reached equilibrium at about 80 percent of the initial concentration injected. The oxygen consumption peaked at about 40 kg/day during the initial infiltration period. Levels of cyanide, a contaminant identified after the treatment began, also decreased, although biodegradation was not shown to be the cause. Total bacterial counts in the groundwater increased tenfold, but bacteria potentially harmful to man did not increase. The drinking water from this aquifer contained no trace of contaminants after one and a half years of ozone treatment.

Saturated aliphatic compounds that do not contain easily oxidized functional groups are not readily reactive with ozone; for example, saturated aliphatic hydrocarbons, aldehydes, and alcohols (Sims and Bass, 1984). Reactivity of aromatic compounds with ozone is a function of the number and type of substituents. Substituents that withdraw electrons from the ring deactivate the ring toward ozone; for example, halogen, nitro, sulfonic acid, carbonyl, and carboxyl groups. Substituents that release electrons activate the ring toward ozone; for example, alkyl, methoxyl, and hydroxyl.

The following reactivity patterns with ozone are:

1. phenol, xylene > toluene > benzene

2. pentachlorophenol < dichloro-, trichloro-, tetrachlorophenol

The relatively rapid decomposition rates of ozone in aqueous systems, especially in the presence of certain chemical contaminants or other agents that catalyze its decomposition to oxygen, preclude its effective application to subsurface waste deposits (Amdurer, Fellman, and Abdelhamid, 1985). The half-life of ozone in groundwater is less than one-half hour (Ellis and Payne, 1984) (about 18 minutes (Environmental Protection Agency, 1985b). Since the flow rates of water are likely to be in inches/hr or less, it is unlikely that effective doses of ozone could be delivered very far for chemical oxidation. However, it has been used successfully to supply oxygen for microbial biodegradation (Rice, 1984).

5.1.2.4.2 Hydrogen Peroxide

Hydrogen peroxide is a weaker oxidizing agent than ozone; however, it is considerably more stable in water (Amdurer, Fellman, and Abdelhamid, 1985). It is used to degrade recalcitrant compounds and modify the mobility of some metals (Sims and Bass, 1984). It can be used to increase oxygen levels in the soil. This can increase microbial activity and degradation of organic contaminants (Nagel, et al., 1982). Hydrogen peroxide could be injected into the contaminated soil above the water table in conjunction with nutrients where it would decompose naturally or by enzymatic action to increase the dissolved oxygen content (Texas Research Institute, Inc., 1982).

It was found that air sparging was able to maintain dissolved oxygen levels of only 1 to 2 ppm in a spill area (Nagel, et al., 1982). However, addition of microbial nutrient (a specially formulated, hydrogen peroxide-based nutrient solution; FMC Aquifer Remediation Systems, Princeton, NJ) raised DO levels to over 15 ppm, thus, establishing the efficiency of hydrogen peroxide-based solutions for supplying increased oxygen levels and, thereby, enhancing

the bioreclamation process. Hydrogen peroxide was selected as the source of oxygen for biodegration at the Kelly Air Force Base, Texas, because it can provide about five times more oxygen to the subsurface than aeration techniques (Wetzel, Davidson, Durst, and Sarno, 1986). The increase in microbial densities in stimulated underground spill sites is probably due to the increased oxygen from the hydrogen peroxide (Wilson, Leach, Henson, and Jones, 1986).

Hydrogen peroxide is a strong oxidant and, therefore, is nonselective (Sims and Bass, 1984). It will act with any oxidizable material present in the soil. This could be a problem because the concentration of natural organic material in the soil would be lowered, causing a reduced sorption capacity for some organics. Thus, the effectiveness of peroxide may be inhibited because it simultaneously increases mobility and decreases possible sorption sites.

Hydrogen peroxide is effective for oxidizing cyanide, aldehydes, dialkyl sulfides, dithionate, nitrogen compounds, phenols, and sulfur compounds (FMC Corporation, 1979). The following chemical groups have incompatible reactions with peroxides (i.e., the reaction products are more mobile) (Sims and Bass, 1984):

Acid chlorides and anhydrides
Acids, mineral, nonoxidizing
Acids, mineral, oxidizing
Acids, organics
Alcohols and glycols
Alkyl halides
Azo, diazo compounds, hydrazine
Cyanides
Dithio carbamates
Aldehydes
Metals and metal compounds
Phenols and cresols
Sulfides, inorganic
Chlorinated aromatics/alicycles

Hydrogen peroxide is more soluble in water than molecular oxygen and may provide more oxygen at specific sites of application (Britton, 1985). The enzymatic decomposition reactions are:

$$2H_2O_2 \text{ ---------►} 2H_2O_2 + O_2$$

$$H_2O_2 + XH_2 \text{ ---------►} 2H_2O + X$$

where X can be NADH, glutathione, or other biological reductants.

Hydrogen peroxide and ozone have been used in combination to degrade compounds that are refractory to either material individually (Nakayama, et al., 1979). There is an ongoing debate as to what oxidant is the best (Rich, Bluestone, and Cannon, 1986). Some believe that hydrogen peroxide is the most efficient way to move oxygen through a formation. Others find that air is the most cost-effective oxidizing agent. On the other hand, proponents of both hydrogen peroxide and ozone use aeration in their bioreclamation systems.

Hydrogen peroxide is reasonably inexpensive, is nonpersistent, and is not likely to represent a serious health hazard, if used properly (Texas Research Institute, Inc., 1982; Britton, 1985). However, it is cytotoxic (3 percent is commonly used as a general antiseptic) and may decompose (by enzymatic catalysis or nonenzymatically by in situ physicochemical processes) before reaching its targeted spill location.

This chemical can be toxic to the microorganisms it is intended to stimulate (Rich, Bluestone, and Cannon, 1986). However, the growth rate of hydrocarbon-utilizing bacteria is not necessarily inhibited by high hydrogen peroxide concentrations (Texas Research Institute, Inc., 1982). Even growth enhancement is sometimes observed. Whether or not a given hydrogen peroxide concentration will be toxic to bacteria depends upon the concentration of the organisms when the H_2O_2 is added. Large populations are more successful at surviving high hydrogen peroxide concentrations than small populations.

Bacteria produce hydrogen peroxide from respiratory processes and have enzymes (hydroperoxidases) to protect against the compound's toxicity (Texas Research Institute, Inc., 1982). Hydrogen peroxide has been shown to be toxic to fresh bacterial cultures at levels greater than 100 ppm, although mature cultures suffered less and could function at levels as high as 10,000 ppm (Texas Research Institute, Inc., 1982). Subsequent experimentation with sand columns inoculated with gasoline and gasoline-degrading bacteria showed that 1.0, 0.5 and 0.25 percent hydrogen peroxide solutions were toxic to the bacteria (Texas Research Institute, Inc., 1983). In another study, using a mixed culture of gasoline degraders, the maximum concentration of H_2O_2 that could be tolerated was 0.05 percent (500 ppm), although by increasing the concentration gradually, the level of tolerance could be raised to 0.2 percent (2000 ppm). Hydrogen peroxide could not be used in batch liquid cultures as in sand columns (Wilson, Leach, Henson, and Jones, 1986). The liquid cultures were extremely sensitive to the compound. This was probably due to the nature of the growth in the two environments: in sand, the organisms would grow as a film with multicellular depth; in liquid, they would be unicellular, unprotected by adjacent cells.

There appears to be a critical H_2O_2:organism ratio, above which the catalase-utilizing protective mechanisms of the organisms are overwhelmed. This ratio may be on the order of 2×10^{10}:1 in a given volume of solution, or it may be expressed as 1 ppm H_2O_2:8.9×10^5 bacteria. Flow of hydrogen peroxide through sand-packed columns inoculated with hydrocarbon utilizers is blocked by bubble formation caused by the decomposition of the hydrogen peroxide, at as low as 0.01 percent (100 ppm) H_2O_2.

Another concern is that the hydrogen peroxide will decompose before it reaches the groundwater and cause the precipitation of iron and manganese oxides and hydroxides (Environmental Protection Agency, 1985b). Much of the decomposition of hydrogen peroxide in soil and groundwater will be due to reactions with iron salts (Haber and Weiss, 1934). Nonenzymatic decomposition can occur in a variety of reactions, including those in the presence of iron salts, known as Fenton chemistry (Fenton, 1894). The following reactions show how different iron salts affect the decomposition of H_2O_2 (Haber and Weiss, 1934).

$$Fe^{++} + H_2O_2 \text{ ----►} Fe^{+++} + OH^- + OH\cdot \text{ (hydroxyl radical)}$$

$$OH\cdot + H_2O_2 \text{ ----►} H_2O + H^+ + O_2\cdot^- \text{ (superoxide radical)}$$

$$O\cdot^- + H_2O_2 \text{ ----►} O_2 + OH^- + OH\cdot$$

$$Fe^{+++} + H_2O_2 \text{ ----►} Fe^{++} + 2H^+ + O_2\cdot^-$$

$$Fe^{+++} + O_2\cdot^- \text{ ----►} Fe^{++} + O_2$$

Most decreases of hydrogen peroxide occur rapidly in the top 5.5 cm of a sand column, with only slight decreases thereafter, which may be due to iron stimulation (Wilson, Leach, Henson, and Jones, 1986). On one hand, the molecular oxygen produced from these reactions would help enhance gasoline biodegradation. On the other hand, iron can cause the hydrogen peroxide to decompose before it reaches the intended site. The decomposition rate of peroxide is also greatly accelerated in the presence of another heavy metal, Cu^{++} (Bambrick, 1985).

There are a number of ways to prevent the hydrogen peroxide from decomposing. Standard practice is to add enough phosphate to the recirculated water to precipitate the iron (Wilson, Leach, Henson, and Jones, 19864). High concentrations of phosphates (e.g., 10 mg/l) can stabilize peroxide for prolonged periods of time in the presence of ferric chloride, an aggressive catalyst (Environmental Protection Agency, 1985b). The stabilizing effect of phosphate is fortuitous, since it is a major nutrient for enhancement of underground biodegradation of gasoline. However, there are problems associated with adding high phosphate concentrations, such as precipitation. Some suppliers add an organic inhibitor that will stabilize the peroxide at a rate appropriate to the rate of infiltration, so the oxygen demand of the bacteria attached to the solids is balanced by the oxygen supplied by decomposing peroxide in the recirculated water (Wilson, Leach, Henson, and Jones, 1986). Dworkin Foster, or a similar medium containing the mineral components for growth (except for a source of carbon and energy), can also stabilize hydrogen peroxide and would be a suitable solution for pumping the material underground without premature decomposition (Britton, 1985).

Enhancement of the microbial population has also been reportedly used to reduce levels of iron and manganese in the groundwater (Hallberg and Martinelli, 1976). The process, known as the Vyrodex method, was developed in Finland and has been used in Sweden and other areas where high levels of the two elements are found in the groundwater. Iron bacteria and manganese bacteria oxidize the soluble forms of iron and manganese to insoluble forms; the bacteria use the electrons adsorbed from the oxidation process as sources of energy. Dissolved oxygen is added to the groundwater to stimulate the bacteria to first remove the iron and then later the manganese. As the iron bacteria population builds up and begins to die, it supplies the organic carbon necessary for the manganese bacteria. The efficiency of the process increases with the number of aerations.

Successful use of hydrogen peroxide requires careful control of the geochemistry and hydrology of the site (Wilson, Leach, Henson, and Jones, 1986). Hydrogen peroxide can mobilize metals, such as lead and antimony; and,

if the water is hard, magnesium and calcium phosphates can precipitate and plug the injection well or infiltration gallery.

Heavy metal control procedures involve techniques that effectively prevent contact between the metals and the peroxide (Bambrick, 1985). This is accomplished by using a chelating agent and silicate. The most effective of the commercially available chelating agents is the pentasodium salt of diethylenetriaminepentaacetic acid (Na_5DTPA). This is a negatively charged compound that can form a ringed structure that alters the reactivity of a positively charged ion. The heavy metal ion is bound by covalent bonds off the nitrogens and ionic bonds off the acetate group and, thus, inhibited from entering into undesirable reactions, e.g., Na_3MnDTPA. The breakdown of peroxide can be decreased substantially using the chelate Na_5DTPA in combination with sodium silicate and $MgSO_4$. The real value of DTPA, even when the metal level is low, is in stabilizing the peroxide liquor solution. In the lab this combination reduces the amount of peroxide decomposed to 55 percent after 2 hr. Without DTPA, 95 percent of the peroxide is useless after 1 hr. These results have also been verified in field tests.

The pH does not strongly influence the rate of hydrogen peroxide decomposition by iron salts in aqueous media (Wilson, Leach, Henson, and Jones, 1986). The pathway of its decomposition depends upon the valence of the iron. Liberation of oxygen with resulting bubble formation should occur in groundwater and soil with high concentrations of ferric iron. Addition of $FeCl_3$ to a pumping solution would be a way to form pockets of oxygen bubbles in a short time for bioreclamation of an underground gasoline spill.

An inexpensive hydrogen peroxide can be produced from a coproduct process, such as converting glucose to gluconic acid with glucose-1-oxidase (Hou, 1982).

5.1.2.4.3 Hypochlorite

Another potential oxidant is hypochlorite (Amdurer, Fellman, and Abdelhamid, 1985). It is generally available as potassium, calcium, or sodium hypochlorite (bleach) and is used in the treatment of drinking water, municipal wastewater, and industrial waste (Environmental Protection Agency, 1985b). It reacts with organic compounds as both a chlorinating agent and an oxidizing agent. Hypochlorite additions may lead to production of undesirable chlorinated by-products (e.g., chloroform) rather than oxidative degradation products. Therefore, the use of hypochlorite for *in situ* treatment of organic wastes is not recommended.

5.1.2.4.4 Other Electron Acceptors

Oxygen can be supplemented with other electron acceptors, such as nitrate (Wilson, Leach, Henson, and Jones, 1986). Nitrate can support the degradation of xylenes in subsurface material (Kuhn, Colberg, Schnoor, Wanner, Zehnder, and Schwarzenbach, 1985). This approach is still experimental but offers considerable promise because nitrate is inexpensive, is very soluble, and is nontoxic to microorganisms, although it is of human health concern. Nitrate itself is a pollutant limited to 10 mg/l in drinking water (Environmental Protection Agency, 1985b). Another study found that neither nitrate nor sulfate as terminal electron acceptors in an anaerobic process is effective on

the types of saturated hydrocarbons found in petroleum (Texas Research Institute, Inc., 1982).

Selection of the appropriate oxidizing agent depends, in part, upon the substance to be detoxified and also upon the feasibility of delivery and environmental safety (Environmental Protection Agency, 1985b). Although there are some compounds that will not react with hydrogen peroxide but will react with ozone or hypochlorite, hydrogen peroxide appears to be the most feasible for in situ treatment.

5.1.2.4.5 Possible Approaches for Supplying Oxygen to the Subsurface (Texas Research Institute, Inc., 1982)

1. Injection of Liquified Gases

The injection of liquid oxygen or liquid air in the soil would utilize existing technology. Intermittent injection of liquid oxygen would produce a high concentration of oxygen, which would slowly diffuse into the surrounding strata. Since oxygen is 10 times more soluble in hydrocarbons than it is in water, the hydrocarbon phase could actually act as an oxygen reservoir to replace the oxygen being consumed in the aqueous phase (Faust and Hunter, 1971). Repeated injections would create a flow through the system, preventing build-up of carbon dioxide. Another technique would have to be used to add additional nutrients. This method would be best in an area where the soil contained abundant nutrients.

2. Injection of Oxygen-releasing Compounds (With Nutrients)

The best material for implementing this approach is hydrogen peroxide (Texas Research Institute, Inc., 1982). Injection should ideally be made over the entire contaminated area, both into the water table and at points just above the water table into the gasoline-bearing soil. Recovery wells toward the center of the spill would help contain it and would aid in the recovery of gasoline as it is released from the soil. It may be possible to set up a recirculating system, whereby produced water is cleaned, fertilized and oxygenated, and reinjected into the water table. A variation would be to use a physical oxygenation technique on the injection water instead of a chemical additive.

The injection of a hydrogen peroxide solution (or highly oxygenated water) would appear to have the greatest impact on the total system, along with an appropriate nutrient solution at different depths in the soil strata and into the water table (Texas Research Institute, Inc., 1982). Injection into the contaminated soil above the water table would continuously bathe the gasoline-contaminated region with oxygenated, nutrient-filled water. A large amount of the residual gasoline would be consumed by the bacteria, and it is possible that the emulsifiers they produce would aid in mobilizing the gasoline into the water table where it could be collected by physical means at the producing well(s). Nutrients and oxygen not utilized in the soil would eventually find their way into the water table, where they could be used for cleanup on that system.

Figure A.5-2 shows a schematic of a spill and cleanup operation (Texas Research Institute, Inc., 1982).

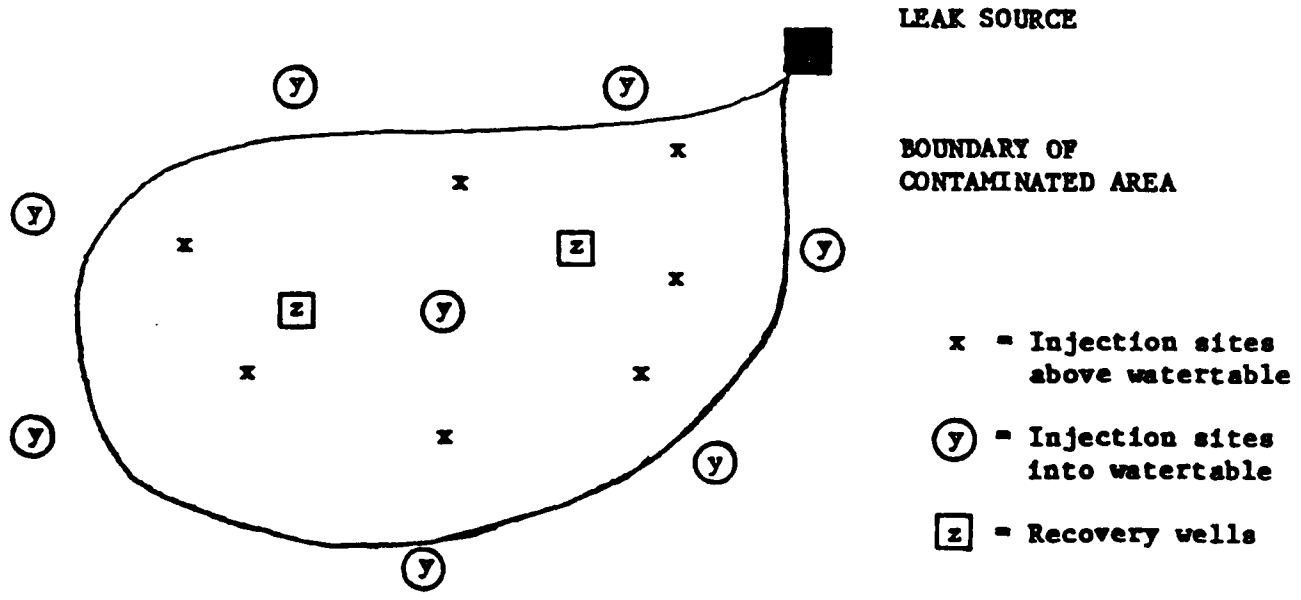


Figure A.5-2. Schematic of a Spill and Cleanup Operation (Texas Research Institute, Inc., 1982)

Since it is expensive to drill a well or injection site, it may be acceptable to have relatively shallow soil injection sites that are just deep enough to get oxygen and nutrients past the plant growth zone so they are not utilized before reaching their intended target (Texas Research Institute, Inc., 1982). If a combination recovery-cleanup operation is employed with the produced water, it could be recirculated throughout the system. Cost-effectiveness would have to be determined for each situation, taking into consideration the cost of transportation to a sewage treatment plant and the cost of bringing in fresh water.

3. Venting

A venting approach would be applicable in an area with porous soil (Texas Research Institute, Inc., 1982). Blowers would be utilized to provide either suction or pressure to create a flow of air through the soil strata. Gaseous ammonia could be added to the input air, supplying nitrogen, but other methods would have to be used for the addition of other nutrients. Also, the effect on groundwater decontamination would be minimal.

In 1981, a technique for soil venting was used in Mont Belvieu, TX, to flush leaked propane and ethane out of the ground (Austin American Statesman, 1980). Liquid nitrogen was pumped underground, and the large volumes of nitrogen gas generated swept the gases through the soil. Possibly, liquid oxygen or air could be utilized in this fashion to supply oxygen to the soil strata. It is not known what effect a stray spark or flame might have on a system such as this. The potential for an underground fire exists.

Costs for the compounds used in the above techniques can be found in Appendix C.

5.1.2.4.6 Commercial Approaches

Enhancing the available oxygen in the soil for microbes to consume hydrocarbon contaminants has improved the rate of degradation (Chowdhury, Parkinson, and Rhein, 1986). Aquifer Remediation System's Bio XL process employs stabilized solutions of hydrogen peroxide (tradename Restore) to increase the amount of oxygen in the soil by more than 25 times, in comparison with air sparging, an earlier method. Another of its products (Restore Microbial Nutrient) prevents precipitation of chemical nutrients. Bioreclamation can now be used in low-permeability formations, where the pumping rate from recovery wells is as low as 5 gal/min.

Groundwater Technology has a similar in situ process, called END (Enhanced Natural Degradation). It is planning to introduce a new system that could cut the amount of hydrogen peroxide consumption by 75 to 90 percent by modifying the oxygen delivery system into a closed loop.

There can be severe oxygen limitation to degradation within inches of the surface of soil (Zitrides, 1983). Polybac Corporation employs tilling of the soil to provide additional oxygen, as well as to better mix a microbial inoculum with the contaminant. Otherwise, the organisms will adhere to the top layers of soil and percolate only slowly to greater depths.

A commercial product (Biostim) produced by Biosystems, Inc., can circulate 500 ppm oxygen in the soil, as opposed to the 10 ppm when air is used as the source of oxygen (Biosystems, Inc., 1986). This is achieved by using "Tysul" WW hydrogen peroxide from Du Pont. It is environmentally safe and is a good source of dissolved oxygen, since the microbes can break down the peroxide into oxygen and water.

5.1.2.4.7 Modifying Soil Oxygen Content for Aerobic Biodegradation

Although some xenobiotic organic compounds appear to require the slow anaerobic metabolism for decomposition, most of these compounds are susceptible to attack by aerobic organisms (Alexander, 1977; Brunner and Focht, 1983). Therefore, assuring the aerobiosis of the soil will enhance the rate of biological decomposition for some compounds. Active microflora have been observed in the top 15 cm of soil, and tilling is suggested as an effective means of promoting aeration (Raymond, Hudson, and Jamison, 1976). Tilling the soil for aeration is common practice in agriculture and has been recommended for hazardous waste-contaminated soil reclamation by practitioners and researchers (Thibault and Elliott, 1979; Arthur D. Little, Inc., 1976). The soil must be tilled at periodic intervals to assure adequate aeration. However, tillage will increase the susceptibility of the site to erosion. Spills often contaminate just the upper layer of soil. Even in these situations, an oxygen limitation on degradation rate can occur, which can be easily solved by tilling (Thibault and Elliott, 1979).

Tilling equipment can aerate surface soils and mix wastes or reagents into the soil (Sims and Bass, 1984). Oxygen must first be dissolved in the interstitial water of the soil, since the viable microorganisms are present in the aqueous environment (Thibault and Elliott, 1979). Therefore, it is important that the contaminated soil be kept moist during the cleanup. It has been concluded that soil moisture is, in itself, a simple and low-cost method of supplying some aeration (Thibault and Elliott, 1980). Oil tilled into the soil immediately and thoroughly after application also significantly reduces the possibility of it being moved hydrologically out of the area (Raymond, Hudson, and Jamison, 1976).

Aeration of subsurface soils not accessible to tillage equipment can be accomplished using construction equipment, such as a backhoe, or a well point injection system, for soils deeper than about 2 ft. Diffusers attached to paint sprayer-type compressors have been used to inject air into a series of 10 wells (Raymond, Jamison, and Hudson, 1976). They deliver about 2.5 cfm to enhance microbial degradation. Various nutrients are added simultaneously. The diffusers are positioned 5 ft from the bottom of the well and below the water table. Aeration through well points has been primarily used for saturated soils and has been shown to be effective for delivering oxygen to the subsurface. It is uncertain whether the technique would also work for unsaturated soils.

If the site is too wet, a drainage system should be installed (see Appendix A, Section 5.1.2.1). Soils with high water tables can be drained using common agricultural techniques. Controls should be set up to prevent run-on and run-off of precipitation (Thibault and Elliott, 1979).

Site geology, in most cases will determine the methods of aeration to be used in a given situation (Raymond, Jamison, and Hudson, 1976). For example, in a fractured dolomite and clay formation, lack of homogeneity makes well injection and distribution of oxygen difficult.

5.1.2.4.8 Modifying Soil Oxygen Content for Anaerobic Biodegradation

Oxygen levels can be decreased by compacting the soil or by saturating the soil with water (Sims and Bass, 1984). By reducing pore sizes and restricting aeration, anaerobic microsite frequency in the soil will increase. Compaction helps draw moisture to the soil surface. This lessens the problems of leaching that may occur, if anaerobiosis is achieved by water addition. Volatilization may also be suppressed by surface soil compaction. Water may still have to be added to achieve the required degree of anaerobiosis; however, it would be less than for an uncompacted soil, also minimizing the leaching potential. After the available oxygen in the water is depleted, the saturated conditions would prevent percolation of additional oxygen to the submerged area. Diking is a common agricultural practice that may be applicable to decreasing the soil oxygen content (Arthur D. Little, Inc., 1976). This would establish and maintain anaerobic conditions as long as the land is kept under water. Another possible method of rendering the site anaerobic would be to add excessive amounts of easily biodegradable organics so the oxygen would be depleted (Environmental Protection Agency, 1985b).

5.1.2.5 Nutrients

Addition of nutrients to hydrocarbon-contaminated surface soil has proved useful in increasing microbial degradation. The total numbers of microbes increase greatly after a petroleum spill. An increase was noted from 10^6 to 10^8 organisms/g after an oil well blowout (Odu, 1972). Application of fertilizer stimulated greater microbial growth and utilization of some components of oil (Westlake, Jobson, and Cook, 1978). Low concentrations of readily metabolized organic compounds (peptone, calcium lactate, yeast extract, nicotinamide, riboflavin, pyridoxine, thiamine, ascorbic acid) often promote the growth of the oxidizer, but high concentrations will retard the degradation of the hydrocarbons (Zobell, 1946; Morozov and Nikolayov, 1978).

Nitrogen and phosphate are the nutrients most frequently present in limiting concentrations in soils (Environmental Protection Agency, 1985b). Other nutrients required for microbial metabolism include potassium, magnesium, calcium, sulfur, sodium, manganese, iron, and trace metals. The elements essential for biological growth and sources for them are presented in Table A.5-7 (Mattingly, 1975).

The nitrogen requirement value (NRV) is the amount of nitrogen required by microorganisms to decompose/degrade a particular organic chemical waste (Parr, Sikora, and Burge, 1983). It depends mainly upon two factors: the chemical composition of the waste and the rate of decomposition. This value is also affected by the other soil factors.

After contact with an oily waste, microbial activity initially decreases (Hornick, Fisher, and Paolini, 1983). This may be due to the same initial decrease in mineral nitrogen resulting from nitrogen immobilization by hydrocarbon-metabolizing microbes using up all available nitrogen. In time,

Table A.5-7. Essential Elements for Biological Growth (Based on Requirements for Plant Growth) (Mattingly, 1975)

Elements	Source
Major nutrients	
Carbon	Air and water
Hydrogen	
Oxygen	
Nitrogen	Soil, inorganic fertilizers, or in waste
Phosphorus	
Potassium	
Sulfur	
Calcium	Soil liming materials, or in waste
Magnesium	
Minor nutrients	
Iron	Soil, soil amendments, or in waste
Manganese	
Boron	
Molybdenum	
Copper	
Zinc	
Chlorine	
Sodium	
Cobalt	
Vanadium	
Silicon	

the microorganisms will adapt to the high C:N ratio and increase the total microbial population (Overcash and Pal, 1979).

Phosphorus concentrations in the soil solution are usually low, ranging from 0.1 to 1 ppm, since this element is mostly associated with the solid phase in soils (Hornick, 1983). In acid soils, phosphorus reacts with iron and aluminum hydroxides to produce adsorbed forms of phosphorus that are in equilibrium with the soil solution or are precipitated and, thus, occluded by the minerals. At low concentrations in calcareous soils, phosphorus is adsorbed onto calcium carbonate; at high concentrations, calcium phosphate minerals are formed (Mattingly, 1975). The common forms of phosphorus in soil are $H_2PO_4^{-2}$ in basic soil solutions.

The quantity of nitrogen and phosphorus required to convert 100 percent of the petroleum carbon to biomass may be calculated from the C:N and C:P ratios found in cellular material (Dibble and Bartha, 1979a). Accepted values for a mixed microbial population in the soil are: C:N, 10:1 (Waksman, 1924); and C:P, 100:1 (Thompson, Black, and Zoellner, 1954). In reality, a complete assimilation of petroleum carbon into biomass is not achievable under natural conditions. Some of the petroleum compounds are recalcitrant or are metabolized slowly over long periods. From petroleum compounds that are readily metabolized, some carbon will be mineralized to carbon dioxide. Thus, efficiency of conversion of substrate (petroleum) carbon to cellular material is less than 100 percent. The optimal C:N and C:P ratios are expected to be wider than the theoretical values.

Under most growth conditions, about half of the carbon available from growth hydrocarbons eventually becomes cellular biomass (Texas Research Institute, Inc., 1982). This consists primarily of proteins, nucleic acids, amino acid, purines, pyrimidines, lipids, and polysaccharides.

Measurement of soil organic carbon, organic nitrogen, and organic phosphorus allows the determination of its C:N:P ratio and an evaluation of nutrient availability (Sims and Bass, 1984). If the ratio of organic C:N:P is wider than about 300:15:1 (100:15:13, Thibault and Elliott, 1980; 100:15:3, Thibault and Elliott, 1979), and available (extractable) inorganic forms of nitrogen and phosphorus do not narrow the ratio to within these limits, supplemental nitrogen and/or phosphorus should be added, such as by addition of commercial fertilizers (Kowalenko, 1978). One such product is POLYBAC^{r} N Biodegradable Nutrients, which contains the proper balance of nitrogen and phosphorus required in a form readily available for microbial uptake (Thibault and Elliott, 1979).

The ratio depends upon the rate and extent of degradation of the chemicals involved (Josephson, 1983) and may vary according to the particular contaminant present. Biodegradation of complex oily sludges in soil occurs most rapidly when nitrogen is added to reduce the carbon to nitrogen (C:N) ratio to 9:1 (Brown, Donnelly, and Deuel, 1983). That of petrochemical sludge is most rapid when nitrogen, phosphorus, and potassium are added at a rate of 124:1, C:NPK.

During experiments on landfarming waste oil, it was determined that carbon-to-nitrogen and carbon-to-phosphorus ratios of 60:1 and 800:1, respectively, were optimal under the conditions used (Dibble and Bartha, 1979a). Addition of yeast extract or domestic sewage did not prove beneficial.

Urea formaldehyde was found to be the most satisfactory nitrogen source tested, since it effectively stimulated biodegradation and did not leach nitrogen, which could contaminate the groundwater (Dibble and Bartha, 1979b). A problem with this technology is that run-off water from the site could contain high amounts of oil and fertilizer (Kincannon, 1972).

In one situation, the addition of sodium nitrate enhanced the oxidation of hydrocarbons and the ultimate decay of the resulting organic carbon compounds to inorganic carbon compounds (Dietz, 1980). Addition of potassium orthophosphates, KH_2PO_4 and K_2HPO_4, had no effect on biodegradation in this application. The phosphate precipitated in a very early stage, due to the presence of calcium. Calcium promotes flocculation, the clumping of tiny soil particulates, and may prevent thorough incorporation of phosphate into the soil (Brady, 1974).

Microorganisms may be limited by phosphorus but not nitrogen (Thorn and Ventullo, 1986). Neither nitrogen nor phosphorus enrichment alone stimulated the biodegradation of phenol in topsoil (Atlas and Bartha, 1973). However, in two different types of subsurface soils, addition of these nutrients significantly stimulated mineralization. Phosphorus enrichment had the greatest effects, and the effects of simultaneous nitrogen and phosphorus amendments were similar to those observed with phosphorus alone. Phosphorus limitation may be widespread in subsurface soils. If the input of phosphorus into the subsurface is disproportionate to that of organic compounds, phosphorus limitation could greatly reduce the ability of microbes in the lower soil profile to attenuate pollutants during their transit to the underlying groundwater, assuming oxygen or another electron acceptor is not limiting. It should be noted that addition of phosphates can result in the precipitation of calcium and iron phosphates (Environmental Protection Agency, 1985b). If calcium concentrations are high, the added phosphate can be tied up by the calcium, and would, therefore, not be available to the microorganisms.

If calcium is present at 200 mg/l, it is likely that calcium supplementation is unnecessary (Environmental Protection Agency, 1985b). Calcium deficiencies usually occur only in acid soils and can be corrected by liming (JRB Associates, Inc., 1984b). If the soil is deficient in magnesium, the use of dolomitic lime is advised. It is desirable to have a high level of exchangeable bases (calcium, magnesium, sodium, and potassium) on the surface exchange sites of the soil for good microbial activity and for preventing excessively acid conditions. Sulfur levels in soils are usually sufficient; however, sulfur is also a constituent of most inorganic fertilizers. Micronutrients are also present in adequate amounts in most soils.

Key trace elements are also essential to the stimulation of bacterial growth (Kincannon, 1972). These are required in such small doses that most are already abundant in the soil. They include sulfur, sodium, calcium, magnesium, and iron. Copper, zinc, and lead are normally considered to exhibit harmful effects on biological growth. Addition of yeast cells can serve as a nutrient source (Lehtomakei and Niemela, 1975). Organic and inorganic nutrients in natural waters affect the rate of mineralization of organic compounds in trace concentrations (Kaufman, 1983). Inorganic nutrients, arginine, or yeast extract often enhanced, but glucose reduced, the rate of mineralization.

The concentration of nutrients and organics should be kept as uniform as possible to protect against shock loading (Environmental Protection Agency, 1985b). Nitrogen must be applied with caution to avoid excessive application (Saxena and Bartha, 1983). Nitrate or other forms of nitrogen oxidized to nitrate in the soil may be leached to the groundwater (nitrate is itself a pollutant limited to 10 mg/l in drinking water (Environmental Protection Agency, 1985b). Some nitrogen fertilizers may also tend to lower the soil pH, necessitating a liming program to maintain the optimal pH for biological activity. Low concentrations of readily metabolized organic compounds (peptone, calcium lactate, yeast extract, nicotinamide, riboflavin, pyridoxine, thiamine, ascorbic acid) often promote the growth of the oxidizer, but high concentrations will retard the degradation of the hydrocarbons (Zobell, 1946; Morozov and Nikolayov, 1978). The quantity of organic material to add must be determined in treatability studies (Sims and Bass, 1984). Nutrient formulations should be devised with the help of experienced geochemists to minimize problems with precipitation and dispersion of clays (Environmental Protection Agency, 1985b). Special soil preconditioners and nutrient formulations to reduce these problems and maximize nutrient mobility and solubility are being investigated.

Population turnover allows for the recycling of nutrients (Dibble and Bartha, 1979a). However, it is expected that fertilizer in the optimal ratios will have to be reapplied, as necessary. The best fertilizers for soil application are in a form of readily usable nitrogen and phosphorus and also in a slow-release form to provide a continuous supply of nutrients, which is beneficial in terms of fertilizer savings and minimized leaching from the oil-soil interface (Atlas, 1977).

A liquid fertilizer containing: 3340 lb ammonium sulfate, 920 lb disodium phosphate, and 740 lb monosodium phosphate was injected into wells at a contaminated site at in Marcus Hook, PA (Raymond, Jamison, and Hudson, 1976). Addition of nutrients in this form accelerated the removal of contaminating gasoline.

There are many substances that would be suitable as fertilizers, and their compositions and origins differ considerably (Sims and Bass, 1984). The choice of an appropriate fertilizer can be complicated, and an agronomist should be consulted to develop a fertilization plan at a hazardous waste site. A plan may include types and amounts of nutrients, timing and frequency of application, and method of application. The nutrient status of the soil and the nutrient content of the wastes must be determined to formulate an appropriate fertilization plan.

An optimum fertilization program has been proposed (Kincannon, 1972). Chemicals are added so as to attain a slight excess of nitrogen, phosphorus, and potassium in the contaminated area. In addition, soil testing for ammonia and nitrates is conducted at regular monthly intervals. Small doses of ammonium nitrate are added, as needed, to maintain the ammonium or nitrate surplus. Urea is used as a nitrogen source to avoid the initial increase in soil salts, which may result from additions of other fertilizer stocks. Ammonium nitrate is subsequently applied, once urea is deemed no longer necessary. Potash is added as a potassium source.

An application method must also be selected. In agricultural application, fertilizers are either applied evenly over an area or concentrated at given points, such as banded along roots. However, at a hazardous waste site, fertilizer will likely be applied evenly over the whole contaminated area and incorporated by tilling, if necessary. Nutrients can also be injected through well points below the plow layer.

With broadcast fertilization, the fertilizer can be left on the surface or incorporated with a harrow (2 to 3 cm deep), a cultivator (4 to 6 cm deep), or a plow (a layer at bottom of furrow; e.g., 15 cm deep). The depth depends upon the solubility of the fertilizer and the desired point of contact in the soil. In general, nitrate fertilizers move freely, while ammonia nitrogen is adsorbed by soil colloids and moves little until converted to nitrate. Potassium is also adsorbed and moves little except in sandy soils. Phosphorus does not move in most soils. Therefore, potassium and phosphorus need to be applied or incorporated to the desired point of use.

Site geology, in most cases, will determine the methods of fertilization to be used in a given situation (Raymond, Jamison, and Hudson, 1976). For example, in a fractured dolomite and clay formation, lack of homogeneity makes well injection and distribution of nutrients difficult. Use of diammonium phosphate could result in excessive precipitation, and nutrient solution containing sodium could cause dispersion of the clays, thereby, reducing permeability (Environmental Protection Agency, 1985b). High calcium could cause precipitation of added phosphate, rendering it unavailable to microbial metabolism. If a site is likely to encounter problems with precipitation, iron and manganese addition may not be desirable. If the total dissolved solids content in the water is extremely high, it may be desirable to add as little extra salts as possible.

Earthworms also contribute to the degradation of organic materials in soil (Hornick, 1983). As a result of their movement, they carry nutrients to deeper soils and improve soil aeration. Macrofauna, in general, play an essential role in the decomposition of wastes, and the addition of materials to the soil that are toxic to these organisms can alter the rate of decomposition.

Results of oil biodegradation in Marcus Hook, PA, and Corpus Cristi, TX, indicated that fertilizer was not a factor in biodegradation until approximately 50 percent of the oil had been degraded (Raymond, Hudson, and Jamison, 1976). However, other environmental factors may have affected these studies, and this cannot be regarded as conclusive.

A large kerosene spill (1.9 million l) in New Jersey was cleaned up with enhancement (Dibble and Bartha, 1979c). Much of the kerosene was recovered by physical means and by removing 200 m^3 of contaminated soil. Following stimulation of microbial degradation by liming, fertilization, and tillage, phytotoxicity was reduced.

Addition of nitrogen and phosphorus fertilizer at another site resulted in a doubling of the oil biodegradation rate of 70 bbl/acre/month to as much as 1.0 lb/ft^3/month (Kincannon, 1972). It is recommended that monthly determinations of nitrogen and phosphorus levels in the soil and periodic fertilizer application, when necessary, will optimize the fertilization process. The cost of soil disposal of oily wastes was estimated at $3.00/bbl.

Degradation rates of up to 100 bbl/acre/month were reported, when the oil was applied to fertilized soils (Francke and Clark, 1974).

5.1.2.6 Organic Matter

Organic matter is generally an amorphous organic residual in soils, which, when present in sufficient amounts, has a beneficial effect on the physical and chemical properties of the soil because of its high cation exchange capacity (the total amount of cations held exchangeably by a unit mass or weight of a soil), high specific or reactive surface, and large amounts of exchangeable bases (Hornick, 1983). Humic substances (humic acids, fulvic acids, and humins; National Academy of Sciences, 1977) constitute 60 to 80 percent of the total organic content of most groundwaters and sediments (Khan, 1980). These organics tend to be recalcitrant to degradation. The crude humin consists of humic acid and hymatomelanic acids containing functional carboxyl and phenolic hydroxyl groups responsible for exchange and adsorption reactions (Hornick, 1983). Both humates and fulvates show a high degree of reactivity due to their acidic functional groups. The reaction of these materials with cations in the soil solution is strongly pH dependent.

Other organic materials involved in metal reactions and complexation in soils are plant root exudates and various degradation products, which can serve as the base for the humic fraction of the soil (Hornick, 1983). Easily decomposed organic contaminants can become part of an important soil process and result in a substantial increase in beneficial organic materials. It is likely that maintaining a supply of biodegradable organic matter in site soils would allow a higher population of diverse microbes capable of degrading many kinds of toxic organic compounds (Kaufman, 1983).

Humus increases the water-holding capacity of soil by swelling when wet to absorb two to three times its weight in water (Hornick, 1983). Because of its surface area, surface properties, and functional groups, humified soil can serve as a buffer, an ion exchanger, a surfactant, a chelating agent, and a general sorbent to help in the attenuation of hazardous compounds in soils (Ahlrichs, 1972). Enzyme activities of soil organisms can be responsible for coupling xenobiotic compounds and their breakdown products to soil humic materials (Bollag, 1983). Bound hazardous organic compounds, including toxic metabolites, should be monitored. Humus-bound xenobiotic compounds may be slow to mineralize or be transformed to innocuous forms (Khan, 1982), and they may become an integral part of the soil matrix. In these cases, the humic content of the soil should probably not be increased. Hazardous materials bound to humus might be released by microbial action and be subject to leaching, volatilization, or reattachment to soil organic matter. This suggests that treatment is not complete until it can be demonstrated that these compounds are absent or at a safe level in the soil (Morozov and Nikolayov, 1978).

Natural organic matter can be added to the soil, such as in the form of synthetic commercial organics, cattle manure, sewage sludge, or crop residues (Arthur D. Little, Inc., 1976). Commercial synthetic organics are expensive and their suitability for microbial growth is uncertain. Sewage sludge and cattle manure are the least expensive supplements; however, their use is limited since they contain variable quantities of trace elements that may disturb the expected soil mechanisms for degradation. They also contain populations of organisms, which, although they are usually enteric and do not

survive long in the soil, may represent enough competition to slow the build-up of the desired soil microorganisms. Eight tons/acre of alfalfa meal has been shown to be as effective in stimulating microorganisms as 80 tons/acre of cattle manure. Considerable energy source is removed in the digestive tract of the cattle. The addition of organic wastes, such as animal manure and sewage sludge compost, decreases soil bulk density and increases infiltration and permeability, since organic wastes tend to increase soil aggregation and porosity (Hornick, Murray, and Chaney, 1979).

Mixed results have been obtained by different researchers with using manure amendments to increase the rate of degradation of organic chemicals. While some workers reported that manure increased the rate of degradation of 10 organic chemicals tested, Doyle, 1979, found that manure did not significantly reduce the degradation of any chemical examined. The breakdown of several compounds was positively correlated with the increased total microbial activity of manure-amended soil. Sewage sludge, however, enhanced the breakdown of only two compounds, while decreasing the rate of degradation of nine others.

Some advantages of using municipal sludges in organic waste treatment are that they contain active indigenous populations of microorganisms with degradative potential and they provide necessary nutrients for biodegradation (Sims and Bass, 1984). However, high levels of heavy metal will adversely affect this population.

Nonspecific, readily biodegradable organic matter should be added and mixed into the soil as dry materials or as slurries (Sims and Bass, 1984). Straw has been added to soils to increase adsorption of s-triazine herbicides (Walker and Crawford, 1968). Fungal mycelium and baker's yeast also improved soil sorption, with nonliving cells exhibiting greater sorption capacity than living cells (Shin, Chodan, and Wolcott, 1970; Voerman and Tammes, 1969). The soil moisture level should be optimized when adding organic matter, and frequent mixing is required to maintain aerobic conditions (Sims and Bass, 1984). Controls to manage the run-on and run-off from the site, as a result of tillage, are necessary to prevent drainage and erosion problems (Kowalenko, 1978). Retreatment may be necessary at intervals as nutrients are used up. The potential achievable level of treatment ranges from low to high, depending upon the solubility, sorption, and biodegradability of the organic constituents.

Hazardous constituents may be initially bound to organic materials, but later released as organic materials decompose (Sims and Bass, 1984). The formation of organo-metal complexes through the organic matter chelation of metals is an important factor governing metal availability (Schnitzer and Khan, 1978). In waste-amended soils, the addition of high amounts of organic matter ensures a predominance of organic matter reactions. The mobility of heavy metals added by wastes is related to the organic matter content of soils, pH, hydrous oxide reactions, and the oxidation-reduction or redox potential of a soil.

Addition of organic material and maintenance of aerobic conditions can result in the oxidation of arsenite to arsenate (Sims and Bass, 1984). Further treatment with ferrous sulfate will form highly insoluble $FeAsSO_4$. Anaerobic conditions must be avoided with this technology to prevent the reduction and

methylation of arsenic to volatile forms, although anaerobic microsites can probably not be completely avoided even in carefully managed soils.

It is generally accepted that subsurface microbes are oligotrophic (Wilson, McNabb, Balkwill, and Ghiorse, 1983); however, in one study, carbon (cellulose) enrichment had little effect on mineralization of phenol in any soil examined (Thornton-Manning, Jones, and Federle, 1987). This response could have been due to the recalcitrance of the added carbon source or inorganic nutrient limitation. The most extensive mineralization occurred in a surface soil, which had the lowest content of organic matter.

It may be necessary to conduct laboratory experiments to determine the biochemical fate of given hazardous compounds in organically enriched soil or compost, (Kaplan and Kaplan, 1982).

Terrestrial oil spillages will probably result in the death of plants, releasing large amounts of nonhydrocarbon organic matter into soil, which might serve as an alternate source of carbon for heterotrophic microorganisms, thereby, interfering with the degradation of the contaminants (Atlas, 1977).

5.1.2.7 Oxidation-reduction Potential

Table A.5-8 shows a succession of events in development of anaerobic conditions, which can occur in water-logged soils or poorly drained soils receiving excessive loadings of organic chemical wastes or crop residues.

Table A.5-8. Succession of Events Related to the Redox Potential (JRB Associates, Inc., 1984b; Takai and Kamura, 1966)

Period of Incubation	System	Redox Potential (mv)	Nature of Microbial Metabolism	Formation of Organic Acids
Early	Disappearance of O_2	+600 to +400	Aerobes	None
	Disappearance of NO^{-3}	+500 to +300	Facultative	Some accumulation
	Formation of Mn^{+2}	+400 to +200	anaerobes	after addition of
	Formation of Fe^{+2}	+300 to +100		organic matter
Later	Formation of S^{-2}	0 to -150	Obligate anaerobes	Rapid accumulation
	Formation of H_2	-150 to -220		Rapid decrease
	Formation of CH_4	-150 to -220		

Oxygen levels in aquatic surface and subsurface environments can also be expressed in terms of the logarithm of the electron concentration pe (Bitton and Gerba, 1985). Values for Eh and pe for various microbiological processes are (at 25°C and pH7):

Process	pe	Eh (in mV)
Aerobic respiration	+13.75	+810
Denitrification	+12.65	+750
Sulfate reduction	- 3.75	-220
Methane formation	- 4.13	-240

The following classification of oxygen levels in soils, based upon their redox potential at pH 7, has been proposed (Patrick and Mahapatra, 1968):

Soil Type	Redox Potential
Oxidized soil	>400 mV
Moderately reduced soil	100 to 400 mV
Reduced soils	-100 to 100 mV
Highly reduced soils	-300 to -100 mV

It may be feasible in some cases to intentionally enhance reducing conditions in the subsurface, thereby, lowering the redox potential (Environmental Protection Agency, 1985b). The pH can be adjusted with the addition of dilute acids or bases.

5.1.2.8 Attenuation

The mixing of indigenous soil layers, or the addition of exogenous soil to contaminated soil, is a means of increasing the extent of immobilization of chemical contaminants at hazardous waste sites (Sims and Bass. 1984). This may also aid in decreasing toxicity of the contaminated soil to soil microorganisms due to high concentrations of constituents, to bring the concentrations to levels that can be successfully biodegraded.

This treatment is applicable to all organic wastes. However, organics that are very soluble in water may be more effectively treated by other methods, since large amounts of soil may be required to reduce the mobility of the compound. If very toxic components are present in the waste, destructive treatment would be the preferable treatment alternative.

The indigenous soil profile is tilled to mix uncontaminated soil with the contaminated layers, importing soil or clay, if necessary (Sims and Bass, 1984). The ease of use of this method depends upon site/soil trafficability considerations for tillage and incorporation of added material. Tillage may cause erosion. The level of attenuation achievable is potentially high with suitable size, soil, and waste characteristics. The mixing of new material may

alter the properties of the natural soil; thus, the effectiveness of this may vary for different compounds and may not be as expected. However, this method should be reliable under most conditions. There is limited field experience in this technology.

5.1.2.9 Texture and Structure

5.1.2.9.1 Soil Texture

Soil composition influences infiltration rate and permeability, water holding capacity, and adsorption capacity for various waste components (Hornick, 1983). Clay soils have a greater capacity for physicochemical attenuation of contaminants than coarse sands or fissured rocks (Pye and Patrick, 1983). A predominance of clay and silt particles in finer textured soils results in a very small pore size, with a slow infiltration rate of water (Hornick, 1983). The presence of montmorillonite, with high shrink-swell tendencies, would cause swelling of the soil with added moisture or water and block any further water movement. Run-off or flooding could then occur, and anaerobic conditions would be induced. Coarse soils of sand and gravel have large interconnecting pores and allow rapid water movement. However, if such a site is excessively drained, nutrients in added material will move too rapidly to be sufficiently adsorbed on the soil. The groundwater can be contaminated, if there is no restrictive layer between the coarse layer and the water table.

5.1.2.9.2 Bulk Density

This is a measure of dry soil weight per unit volume and determines pore space through which water can move (Hornick, 1983). The frequent use of heavy machinery to either work the soil or apply wastes compacts the soil and, thus, increases the bulk density.

5.1.2.9.3 Water-holding Capacity

This capacity is directly related to the soil's bulk density and texture (Hornick, 1983). Soils with very fine or very coarse textures or high bulk densities can not maintain an adequate supply of water: the water content determines available oxygen, redox potential, and microbial activity of a soil system.

5.1.3 Alteration of Organic Contaminants

5.1.3.1 Addition of Surfactants

Spontaneous dissolution rates are important factors affecting the rates of biodegradation (Thomas, Yordy, Amador, and Alexander, 1986). Growth of pure cultures of bacteria on naphthalene, phenanthrene, and anthracene is fastest on the solid substrates with the highest water solubilities (Wodzinski and Johnson, 1968). The rate of dissolution of compounds, such as naphthalene, is directly related to their surface areas (Thomas, Yordy, Amador, and Alexander, 1986), and increasing the surface area of hexadecane increases its microbial destruction (Fogel, Lancione, Sewall, and Boethling, 1985). Degradation of polychlorinated biphenyls by a Pseudomonas sp. is enhanced when the surface area of the substrate is increased by emulsification (Liu, 1980). In fact, the first step in hydrocarbon assimilation by Candida lipolytica is the microbial

enhancement of the solubilization of the substrate (Goma, Pareilleux, and Durand, 1974).

This principle can be utilized to improve the degradation of organic compounds in contaminated sites. If it is inconvenient, expensive, or too time-consuming to supply oxygen or other electron acceptors to organisms metabolizing oily contaminants in situ, it might be possible for the subsurface organisms to emulsify the hydrocarbons, if conditions are suitable (Wilson, Leach, Henson, and Jones, 1986). Use of bioemulsification of oils for microbial enhancement of oil recovery from petroleum reservoirs (Cooper, 1982) should be directly applicable to petroleum product spills. Bacteria from a well contaminated by a spill of JP-5 jet fuel could emulsify the fuel, if the well water was supplemented with phosphate and nitrate (Ehrlich, Schroeder, and Martin, 1985). The surfactants not only emulsify the hydrocarbons, but also aid in mobilizing them through soil and water (Vanloocke, Verlinde, Verstraete, and DeBurger, 1979). In favorable geological situations, the mobile emulsions could be removed by pumping for treatment on the surface (Ehrlich, Schroeder, and Martin, 1985).

Detergents are able to increase microbial membrane permeability (Gloxhuber, 1974), and substances from humic acids may have the same effect (Visser, 1982). The addition of humic products to a culture medium resulted in a two thousandfold increase in growth (Visser, 1985). These substances appear to induce a change in metabolism, allowing the organisms to proliferate on substrates they could not previously utilize. Tween 20-80 and Brij 35 increased microbial ATP levels, possibly as a result of an increased metabolic rate with the greater amount of nutrients; however, the mechanisms involved with the humic material have not yet been elucidated. It has been recommended that humic products be incorporated in media for determination of microbial activities in terrestrial and aquatic environments. Humic substances constitute the major part of the natural organic constituents of most waters and sediments, typically forming 60 to 80 percent of the total organic content (Khan, 1980).

The removal of petroleum hydrocarbons from soil was improved by orders of magnitude by use of a 2 percent aqueous solution of Adsee 799 (Witco Chemical) and Hyonic NP-90 (Diamond Shamrock) rather than just water washing (Ellis, Payne, and McNabb, 1985). This combination has adequate solubility in water, minimal mobilization of clay-sized soil fines (to maintain soil permeability), good oil dispersion, and adequate biodegradability. It is potentially useful for in situ cleanup of hydrophobic and slightly hydrophilic organic contaminants in soil. Removal efficiency of the latter would be significantly improved by use of aqueous surfactants in soils with high TOC (total organic carbon) values.

It may even be possible to add or select for organisms that produce emulsifiers. Surface active agents are excreted into the aqueous medium when certain organisms are grown on liquid hydrocarbons, particularly n-alkanes (Zajic and Gerson, 1977). When exposed to hydrocarbons, the lipid content of the cell wall increases significantly, which increases the affinity of the microbe for the hydrocarbon (Blanch and Einsele, 1973). The surface active agents are mainly lipids, lipoproteins, or sugar-lipid complexes, which reduce the interfacial tension between the hydrocarbon and the aqueous medium. Emulsions formed are stabilized by the polysaccharide polymers secreted

extracellularly by the microbes. These can be then absorbed through the lipophilic cell wall to be utilized by the microorganism as a carbon and energy source.

A strain of Corynebacterium sp., isolated from sewage sludge, was grown in mineral salts medium with hexadecane (3.0 percent v/v) as a carbon and energy source (Panchal, Zajic, and Gerson, 1979). Both hydrophobic and hydrophilic emulsifiers were isolated from the same culture broth. The lipid extract was a very potent emulsifying agent, while the polysaccharide was very weak, unless used at a high concentration and in combination with Tween 20.

5.1.3.2 Photolysis

The major photoreaction taking place with pesticides in the atmosphere is oxidation (Crosby, 1971) involving the OH^0 radical or ozone, of which the OH^0 radical is the species of greatest reactivity (Lemaire, Campbell, Hulpke, Guth, Merz, Philop, and Von Waldow, 1982). Based on a first-order rate of reaction of vapor phase reactions with the OH^0 radical, the half-life of a specific chemical species can be estimated, if its OH^0 radical reaction rate constant is known using:

$$t_{1/2} = 0.693/k_{OH}{}^{0}[OH^0])$$

where

$t_{1/2}$ = time to decrease component concentration by 50%

$k_{OH}{}^{0}$ = OH radical reaction rate constant (cm^3/molecule)

$[OH^0]$ = atmospheric OH radical concentration (4×10^5 molecules/cm^3)

= 6645×10^{-19} moles /cm^3)

Table A.5-9 presents the OH^0 radical reaction rate constants for various organic compounds (Sims and Bass, 1984). The higher the number, the faster the oxidation of the compounds.

In order to assess the potential for use of photodegradation, the specific compound's atmospheric reaction rate (log $K_{OH}{}^{0}$) and the anticipated reaction products must be known (Crosby, 1971). If a compound is poorly photoreactive (e.g., a $t_{1/2}$ in the atmosphere greater than one day) volatilization suppression may be required to maintain safe ambient air concentrations at the site.

Groups that typically do not undergo direct photolysis include saturated aliphatics, alcohols, ethers, and amines (Sims and Bass, 1984). Photodegradable organic wastes generally include compounds with moderate to strong absorption in the >290-nm wavelength range. Such compounds generally have an extended conjugated hydrocarbon system or a group with an unsaturated hetero atom (e.g., carbonyl, azo, nitro). Enhanced photodegradation of soil contaminants may be accomplished through the addition of various proton donor materials to the contaminated soils.

Table A.5-9. Rate Constants for the Hydroxide Radical Reaction in Air with Various Organic Substances (Sims and Bass, 1984)

k_{OH}^{o} in Units of $(Mole\text{-}sec)^{-1}$

Substance	$\log_{air} k_{OH}^{o}$
Acetaldehyde	9.98
Acrolein	10.42
Acrylonitrile	9.08
Allyl chloride	10.23
Benzene	8.95
Benzyl chloride	9.26
Bis(chloromethyl)ether	9.38
Carbon tetrachloride	<5.78
Chlorobenzene	8.38
Chloroform	7.78
Chloromethyl methyl ether	9.26
Chloroprene	10.44
o-,m-,p-cresol	10.52
p-cresol	10.49
Dichlorobromobenzene	8.26
Diethyl ether	9.73
Dimethyl nitrosamine	10.37
Dioxane	9.26
Epichlorohydrin	9.08
1,2-epoxybutane	9.16
Epoxypropane	8.89
Ethanol	9.28
Ethyl acetate	9.06
Ethyl propionate	9.03
Ethylene dibromide	8.18
Ethylene dichloride	8.12
Ethylene oxide	9.08
Formaldehyde	9.78
Hexachlorocyclopentadiene	10.55
Maleic anhydride	10.56
Methanol	8.78
Methyl acetate	8.04
Methyl chloroform	6.86
Methyl ethyl ketone	9.32
Methylene chloride	7.93
Methyl propionate	8.23
Nitrobenzene	7.56
Nitromethane	8.81
2-nitropropane	10.52
n-nitrosodiethylamine	10.19
Nitrosoethylurea	9.89
n-propylacetate	9.41
Perchloroethylene	8.01

Table A.5-9. Rate Constants for the Hydroxide Radical Reaction in Air with Various Organic Substances (Sims and Bass, 1984) (Continued)

k_{OH}^{o} in Units of $(Mole\text{-}sec)^{-1}$

Substance	$\log_{air} k_{OH}^{o}$
Phenol	10.01
Phosgene	nonreactive
Polychlorinated biphenyls	<8.78
Propanol	9.51
Propylene oxide	8.89
Tetrahydrofuran	9.95
Toluene	9.52, 9.56
Trichloroethylene	9.12
Vinylidene chloride	9.38
o-,m-,p-xylene	9.98

Source: Adopted from (Lemaire, Campbell, Hulpke, Guth, Merz, Philop, and Von Waldow, 1982 and Cupitt, 1980)

Table A.5-10 presents additional constants, with an estimation of the likelihood of a photolysis reaction occurring within the ambient atmosphere (Sims and Bass, 1984).

5.1.3.3 Supplementing Threshold Concentrations of Contaminants

Table A.5-10. Atmospheric Reaction Rates and Residence Times of Selected Organic Chemicals (Sims and Bass, 1984)

Compound	$k_{OH} \times 10^{12}$ (cm^3/molecule/sec^{-1})	Direct Photolysis Probability	Physical Removal Probability	Residence Time (days)	Anticipated Photoproducts
Acetaldehyde	16	Probable	Unlikely	0.03-0.7	H_2CO, CO_2
Acrolein	44	Probable	Unlikely	0.2	OCH-CHO, H_2CO HCOOH, CO_2
Acrylonitrile	2	-	Unlikely	5.6	H_2CO, CN^0, HC(O)CN, HCOOH
Carbon Tetrachloride	<0.001	-	Unlikely	>11,000	Cl_2CO, Cl^0
o-,m-,p-cresol	55	-	Unlikely	0.2	hydroxynitrotoluenes, ring cleavage products
Formaldehyde	10	Probable	Unlikely	0.1-1.2	CO, CO_2
Nitrobenzene	0.06	Possible	Unlikely	190	Nitrophenols, ring cleavage products
2-Nitropropane	55	Possible	Unlikely	0.2	H_2CO, CH_3CHO
Phenol	17	-	Possible	0.6	Dihydroxybenzenes, nitrophenols, ring cleavage products
Toluene	6	-	Unlikely	1.9	Benzaldehyde, cresols, ring cleavage products, nitro compounds
o-,m-,p-xylene	16	-	Unlikely	0.7	substituted benzaldehydes, hydroxy xylenes, ring cleavage products, nitro compounds

Source: (Cupitt, 1980)

5.2 OPTIMIZATION OF GROUNDWATER BIODEGRADATION

Some of the information in this section may duplicate material covered in Section 5.1, Optimization of Soil Biodegradation, and Section 5.3, Optimization of Freshwater, Estaurine, and Marine Biodegradation; however, it is presented here under a separate heading, with other related information, to accommodate those readers who may specifically wish to address treatment of groundwater contamination only.

5.2.1 Biological Enhancement

5.2.1.1 Seeding of Microorganisms

5.2.1.2 Acclimation

Naphthalene could be degraded rapidly in aerobic groundwaters contaminated by PAHs (Lee and Ward, 1984b) or in groundwater near oil and gas beds (Slavnia, 1965). It was biodegraded in an aquifer recharged with reclaimed water from wastewater treatment after an initial lag (Roberts, McCarty, Reinhard, and Schreiner, 1980). These results demonstrate the importance of acclimation of the organisms to the contaminant.

It is possible that adaptation requirements may be different for mineralization and degradation (Swindoll, Aelion, Dobbins, Jiang, Long, and Pfaender, 1988). A microbial community isolated from fine sand aquifer subsoil about 5 ft below the surface was capable of biodegrading xenobiotics. The community appeared to be preadapted to utilization of some of the chemicals. Phenol was initially mineralized at a rapid rate, which leveled off such that a maximum percent respiration of the compound was reached, usually within weeks. While m-cresol, m-aminophenol, and aniline exhibited an initially slow degradation rate, which was maintained throughout the incubation period (7 months), a linear increase in percent mineralized occurred with time. Only p-nitrophenol was found to require an adaptation period, after which the rate of degradation was very rapid.

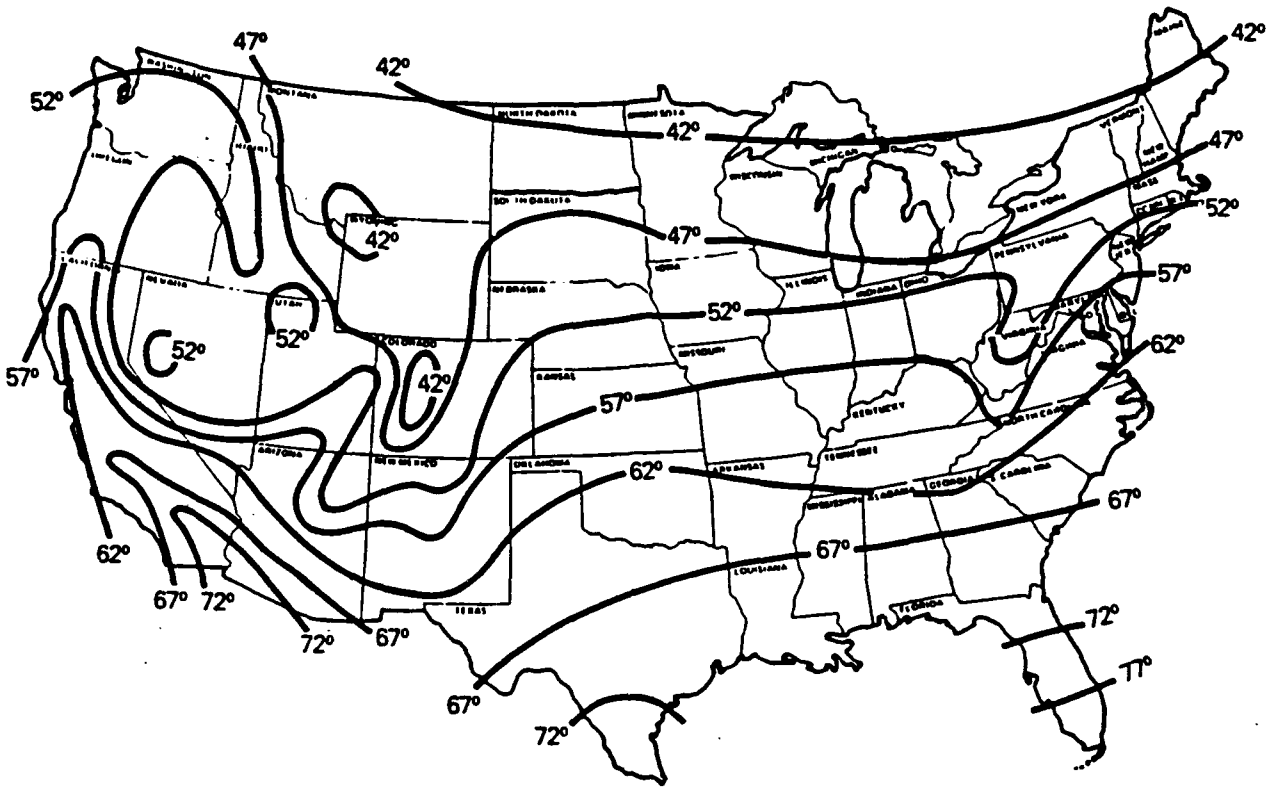
5.2.2 Optimization of Groundwater Factors

5.2.2.1 Temperature

Figure A.5-3 shows typical groundwater temperatures throughout the United States (Environmental Protection Agency, 1985b).

5.2.2.2 Oxygen Supply

Oxygen may be especially limiting in areas of high oil concentration or when oil sinks into sediments (ZoBell, 1969). Oxygen concentration was 3 to 5 mg/l in samples from wells outside the area of jet-fuel contamination, but was zero in wells containing jet fuel (Ehrlich, Schroeder, and Martin, 1985). Evidently, aerobic bacteria using jet fuel as an energy source rapidly consume all the available oxygen. The absence of nitrate and oxygen in the groundwater contaminated by jet fuel suggests that in situ degradation might be enhanced, if additional oxygen and inorganic nitrogen are available. In fact, degradation of a plume of contaminated groundwater consisting of naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, dibenzofuran, and fluorene (1,000 to



Source: Johnson Division, UOP Inc., 1975

Figure A.5-3. Typical Groundwater Temperatures (^{0} at 100-ft. Depth) in the United States (Environmental Protection Agency, 1985b)

100 mg/l) was controlled, not by the rate of utilization of the pollutants by the microorganisms, but by the extent of utilization allowed by the supply of oxygen (Wilson, McNabb, Cochran, Wang, Tomson, and Bedient, 1985).

The solubility of benzene (1780 mg/l) is much greater than the capacity for its aerobic degradation in groundwater (Wilson, Leach, Henson, and Jones, 1986). The concentration of the contaminants is an important factor. Concentrated plumes cannot be degraded aerobically until dispersion or other processes dilute the plume with oxygenated water. High concentrations of organic contaminants in the groundwater will deplete the oxygen and aerobic metabolism will stop. Anaerobic biotransformations may take over, but the hydrocarbon degradation would be very slow.

Air can be added to extracted groundwater before reinjection, or it can be injected directly into the aquifer (Environmental Protection Agency, 1985b). The first method, in-line aeration, involves adding air into the pipeline and mixing it with a static mixer to provide a maximum of 10 mg/l oxygen. This concentration will degrade about 5 mg/l hydrocarbons and would, therefore, provide an inadequate oxygen supply. A pressurized line can increase oxygen concentrations, as can the use of pure oxygen.

The equilibrium oxygen concentration in water increases with increased air pressure according to Henry's Law:

where: $C_L = PH_k$

C_L = concentration of oxygen in liquid (mg/l)

= volume fraction (0.21 for O_2 in air)

P = air pressure (atm)

H_k = Henry's Law Constant for oxygen

The value of Henry's Law Constant is 43.8 mg/l-atmosphere at 68°F (20°C). Pressure increases with groundwater depth at the rate of 0.0294 atmospheres per foot.

The use of <u>in situ</u> aeration wells is a more suitable method for injecting air into contaminated leachate plumes (Environmental Protection Agency, 1985b). In-line or <u>in situ</u> oxygenation systems can achieve higher oxygen solubilities and more efficient oxygen transfer to the microorganisms than conventional aeration. A bank of aeration wells can be installed to provide a zone of continuous aeration through which the contaminated groundwater would flow. Oxygen saturation conditions can be maintained for degrading organics during the residence time of groundwater flow through the aerated zone. The required time for aeration can be derived from bench-scale studies. Residence time (t_r through the aerated zone can be calculated from Darcy's equation using groundwater elevations and hydraulic conductivity, as follows:

$$t_r = (L_a)^2/K(h_1-h_2)$$

where:

t_r = residence time (sec)

K = hydraulic conductivity (ft/sec)

L_a = length of aerated zone (ft)

h_1 = groundwater elevation at beginning of aerated zone (ft)

h_2 = groundwater elevation at end of aerated zone (ft)

Solubilities of oxygen in various liquids are four to five times higher under pure oxygen systems than with conventional aeration (Environmental Protection Agency, 1985b). In-line injection of pure oxygen can impart 40 to 50 ppm of dissolved oxygen to water (Brown, Norris, and Raymond, 1984), which will provide sufficient dissolved oxygen to degrade 20 to 30 mg/l of organic material, assuming 50 percent cell conversion. This oxygen will not be consumed immediately, as the oxygen is from aeration. However, pure oxygen is expensive to use and the oxygen is likely to bubble out of solution (degas) before the microbes can utilize it.

There are various methods for injecting air or pure oxygen (Environmental Protection Agency, 1985b). These include use of pumps, propellers, stirrers, spargers, sprayers, and cascades (Texas Research Institute, Inc., 1982). Air can be sparged into wells using diffusers (e.g., diffusers attached to paint sprayer-type compressors that can deliver about 2.5 cubic feet per minute) (Environmental Protection Agency, 1985b). A blower can also be used to provide the flow rate and pressure for aeration, such as 5 psi pressure in a 10-ft aeration well, with an air flow of 5 cubic feet per minute. The solubility of oxygen is very low, approximately 8 mg/l at groundwater temperatures (Wilson and Rees, 1985). Diffusers that sparge compressed air into the groundwater cannot exceed the solubility of oxygen in water (Lee and Ward, 1986). A newly developed method that holds great promise for introducing oxygen to the subsurface is microdispersion of air in water using colloidal gas aprons (CGA), which creates bubbles 25 to 50 um in diameter (Environmental Protection Agency, 1985b). With selected surfactants, dispersions of CGA's can be generated containing 65 percent air by volume.

An in situ aeration well zone must be wide enough to allow the total plume to pass through, and there must be sufficient air to generate a substantial radius of aeration without causing an air barrier to the flow of groundwater (Environmental Protection Agency, 1985b).

Hydrogen peroxide, which decomposes to form water and oxygen, can supply much greater oxygen levels (Lee and Ward, 1986) and has been used successfully to clean up several spill sites (Environmental Protection Agency, 1985b). Advantages of hydrogen peroxide include:

1. Greater oxygen concentrations can be delivered to the subsurface. Only 100 mg/l H_2O_2 provides 50 mg/l oxygen.

2. Less equipment is required to oxygenate the subsurface. Hydrogen peroxide can be added in-line along with the nutrient solution. Aeration wells are not necessary.

3. Hydrogen peroxide keeps the well free of heavy biogrowth. Such growth and clogging can be a problem in air injection systems.

Although hydrogen peroxide may be cytotoxic at levels as low as 200 ppm, it can be added to acclimated cultures at up to 1,000 ppm (Environmental Protection Agency, 1985b). The remediation at Grange, Indiana, involved adding an initial concentration of 100 ppm, and increasing it to 500 ppm over the course of the treatment. Although the compound may degas above 100 ppm and form air bubbles that block the pores in the aquifer, it may be possible to overcome this limitation by stabilizing the hydrogen peroxide solution (Lee and Ward, 1986).

There is field evidence for enhanced degradation with the use of hydrogen peroxide (Yaniga and Smith, 1984). During air sparging with 100 ppm hydrogen peroxide, the dissolved oxygen concentrations in monitoring wells at a site contaminated by gasoline increased from 4 to 10 ppm. This was accompanied by an increase in the numbers of gasoline-utilizing organisms and a reduction in the size of the gasoline plume and a decrease from 4 to 2.5 ppm hydrocarbon. However, other restoration measures were concurrently being employed.

Ozone is also used for disinfection and chemical oxidation of organics in water and wastewater treatment (Environmental Protection Agency, 1985b), but ozone has the same limitations as hydrogen peroxide (Lee and Ward, 1985). In commercially available ozone-from-air generators, ozone is produced at a concentration of 1 to 2 percent in air (Environmental Protection Agency, 1985b). In bioreclamation, this ozone-in-air mixture could be contacted with pumped leachate using in-line injection and static mixing or using a bubble contact tank. A dosage of 1 to 3 mg/l of ozone can be used to attain chemical oxidation. However, the dosage should not be greater than 1 mg/l of ozone per mg/l total organic carbon; higher concentrations may be deleterious to the microorganisms. At many sites, this may limit the use of ozone as a pretreatment method to oxidize refractory organics, making them more amenable to biological oxidation.

In a hydrocarbon-contaminated area in West Germany, the water was pumped out, treated with ozone, and recirculated to the aquifer via injection wells (Lee and Ward, 1985; Environmental Protection Agency, 1985b). About 1 g of ozone per gram of dissolved organic carbon was added to the groundwater, with a contact time of 4 minutes in the above ground reactor. This increased the dissolved oxygen levels to 9 mg/l, with a residual of 0.1 to 0.2 g of ozone per cubic meter in the treated water. The microbial counts subsequently increased in the wells, with a decrease in dissolved organic carbon and mineral oil hydrocarbons. The ozone may have also reacted with the hydrocarbon for partial destruction of the organics. Oxidizing the subsurface could result in the precipitation of iron and manganese oxides and hydroxides. If this is extensive, the delivery system and possibly even the aquifer could become clogged.

Section 5.1.2.4 of Appendix A provides an in-depth review of the use of ozone and hydrogen peroxide for subsurface aeration. It describes problems

associated with using these oxidants and suggests ways to deal with the problems. One such process, called the Vyrodex method, has been developed specifically to reduce the levels of iron and manganese in groundwater. This is accomplished by the addition of iron and manganese bacteria, and stimulation with dissolved oxygen, to prevent the heavy metals from decomposing the hydrogen peroxide before it can reach its intended site (Knox, Canter, Kincannon, Stover, and Ward, 1968).

In cases where the extent of the pollution is large or the water table extends to a depth where physical removal of contaminated material is totally impractical, alternative methods are used (Wilson, Leach, Henson, and Jones, 1986). One of these methods is construction of one or a series of surface infiltration galleries. These galleries take water that has been treated and recirculate it back through the contaminated unsaturated zone. Oxygen is generally added to the infiltrated water during an in-line stripping process for volatile organic contaminants or through aeration devices placed in the infiltration galleries. Recirculation of the water also facilitates movement of contaminants to the recovery well. The dislodged or solubilized contaminant can be treated in a surface treatment system before the water is reinjected. Controlling the rate of groundwater flow is critical to moving oxygen and nutrients to the contaminated zone and optimizing the degradation process. Silty or shaley materials accept water very slowly. Aquifer flow rates should be sufficiently high so that the aquifer is flushed several times over the period of operation (e.g., twice a year over a three-year treatment period (Environmental Protection Agency, 1985b). The operating period will depend upon the biodegradation rate of the contaminants in the plume and the amount of recycle. If the period of operation is excessively long, for example, more than five years, the operating costs of bioreclamation may outweigh the capital costs of another remedial alternative.

Air sparging in injection wells, in conjunction with nutrient addition, appeared to be a contributing factor in removing free gasoline from the groundwater in a cleanup operation in Millville, NJ (Raymond, Jamison, Hudson, Mitchell, and Farmer, 1978). However, dissolved oxygen measurements from surrounding wells did not reflect the additional oxygen input. This approach attacks the oxygenation problem only from the groundwater perspective. Residual gasoline in the soil above the groundwater will continue to leach into the groundwater with time.

5.2.2.3 Nutrients

5.2.2.4 Oxidation-reduction Potential

Nitrate respiration may be a feasible approach to decontaminating an aquifer (Environmental Protection Agency, 1985b). Denitrification (the reduction of NO_3 to NH_3 or N_2) has been demonstrated to occur in contaminated aquifers. Nitrate respiration was used successfully in the treatment of an aquifer contaminated with aromatic and aliphatic hydrocarbons. Nitrate can be added in-line along with other nutrients and intimate mixing with groundwater can occur. The cost is moderate; only the nutrient feed system and an in-line mixer are required.

Nitrate, however, is a pollutant, limited to 10 ppm in drinking water. Consequently, it may be more difficult to obtain permits for use of nitrate

than for oxygen or hydrogen peroxide. Also, degradation rates under anaerobic conditions are not as rapid, and the substrate range is more limited. There is no reason why nitrate respiration would be a better treatment approach, given the amount of success that has been already demonstrated with aerobic processes.

Many compounds can be transformed under anaerobic conditions, but not aerobically; examples are chloroform, bromodichloromethane, dibromochloromethane, bromoform, and 1,1,1-trichloroethane (McCarty, Rittmann, and Bouwer, 1984). Different redox conditions may also affect the transformation of a compound. For example, chloroform and 1,1,1-trichloroethylene can be degraded under methanogenic conditions but not under denitrification conditions (Bouwer and McCarty, 1983b; Bouwer and McCarty, 1983a).

5.2.3 Alteration of Organic Contaminants

5.2.3.1 Addition of Surfactants

5.3 OPTIMIZATION OF FRESHWATER, ESTAURINE, AND MARINE BIODEGRADATION

Some of the information in this section may duplicate material covered in Section 5.1, Optimization of Soil Biodegradation, and Section 5.2, Optimization of Groundwater Biodegradation; however, it is presented here under a separate heading, with other related information, to accommodate those readers who may specifically wish to address treatment of freshwater, estaurine, and marine contamination only.

5.3.1 Biological Enhancement

5.3.1.1 Seeding of Microorganisms

Mixed marine enrichment cultures have been investigated for treating spilled oil (Atlas and Bartha, 1972c). Mixed enrichments can no doubt degrade a highly complex substrate, such as petroleum, more effectively than any single microorganisms, but the practical use of an enrichment of unknown composition is likely to encounter licensing difficulties because of its potential side effects on marine life. It may be necessary to construct an effective mixed culture from known microorganisms with wide and complementing substrate ranges.

In aqueous environments, added seed organisms are likely to dissipate from the oiled area unless they are added in high enough concentrations and in a metabolic state so as to allow for immediate colonization of the oil. It has also been suggested that seed bacteria could be encapsulated to ensure that they adhere to and remain with the oil in aqueous environments (Azarowicz, 1973).

Several commercial mixtures of microorganisms for seeding have been tested and found to be ineffective for degradation of petroleum in the marine environment, although the literature supplied with the mixtures included claims of effectiveness for cleaning up ocean oil spills (Azarowicz, 1973). Even under optimal temperature, aeration, and nutrient conditions, such treatment of oil on seawater failed to stimulate petroleum biodegradation beyond the rate and extent carried out by indigenous microbial populations. The possibility of seeding a hydrocarbonoclastic bacterium into Arctic saline and freshwater ponds has been investigated, using an organism isolated from an estuarine environment (Spain, Milhous, and Bourquin, 1981). This organism stimulated biodegradation in a saline pond but not in a freshwater pond.

Seeding the salt water pond with an oil-degrading Pseudomonas sp., at the same time as adding fertilizer, resulted in the greatest breakdown of the material (Atlas and Busdosh, 1976). However, seeding of the freshwater pond did not increase degradation above that observed with the fertilizer alone. In both ponds, the oil-degrading population decreased one week after seeding. In the saline pond, the counts recovered and increased greatly over a three-week period. In the freshwater pond, the organism had disappeared after two weeks. Apparently, the seed organism was unable to survive in the freshwater ecosystem. This suggests that it is necessary to have a variety of seed organisms available for biodegradation in different environments.

The normal nutrient content of 100 ml of seawater will support less than 0.1 mg (dry wt) microbial biomass (Atlas and Bartha, 1972b). Any seeding operation should be connected with application of an appropriate fertilizer.

In a nutritionally unfavorable environment, there would be little, if any, benefit from relying on inoculation alone. A hydrophobic binder may achieve the selective retention of the mineral supplements within the oil slick (see Section 5.3.2.3).

Since there are low concentrations of microorganisms in the ocean, seed organisms would have less competition with the indigenous population (Atlas, 1977). Predation is a limiting factor in this environment, to which the seed would also be subjected. Ciliates have been observed to strip yeast and bacterial cells from the surface of oil globules during a microbial bloom following an oil spill. Use of yeast strains that can grow within the oil globule offers additional protection from predators. Many marine isolates of Trichosporon sp. show this capability.

A mixture of hydrocarbonoclastic yeasts was seeded into an estuarine environment (Cook, Massey, and Ahearn, 1973). Only two of these yeasts were able to persist for a long period of time: Candida lipolytica lasted three to five months and C. subtropicalis lasted over one year in freshwater and seven months in estuarine environments. The Candida species that persisted were capable of degrading a wide range of alkanes and alkenes with no adverse ecological side effects and were not pathogenic.

A strain of Arthrobacter has been used for cleaning out oil residues from tanker holds (Rosenberg, Englander, Horowitz, and Gutnick, 1975). The dispersing agent produced by the bacterium was not toxic, but the resulting emulsion was. The toxicity (from polar metabolites from the hydrocarbons) could be reduced considerably by dialysis against seawater.

Encapsulation of seed bacteria may be used to ensure the organisms adhere to and remain with the oil in aqueous environments (Gholson, Guire, and Friede, 1972).

5.3.1.2 Acclimation

Natural microbial populations in water/sediment cores from all freshwater sites tested were able to adapt to more rapidly degrade organic substrates supplied at low concentrations (Spain, Milhous, and Bourquin, 1981). However, none of the estuarine or marine populations adapted. The extent of adaptation depended upon preexposure concentrations, but the relationship was not linear. Adaptation was maximal at 15 days after initial exposure and declined gradually until no longer detectable after 50 days. Adaptation periods were found to vary with substrate concentration and were inversely proportional to the concentration (Steen, Paris, and Latimer, 1981).

5.3.2 Optimization of Aquatic Factors

5.3.2.1 Temperature

The effect of temperature on the rate of degradation depends partly upon the compound. For instance, rates of napthalene and anthracene biodegradation in intertidal sediments increase with increasing temperature (Bauer and Capone, 1985); however, the rate for cresol mineralization in estuarine water does not depend upon temperature (Bartholomew and Pfaender, 1983). In studies on the degradation by a Nocardia sp. of Bunker C, hexadecane, and a hydrocarbon

mixture at 5° and 15°C, a 10°-C decrease in temperature resulted in a 2.2-fold decrease in generation time of the bacteria and a slower degradation rate (Mulkins-Phillips and Stewart, 1974b). The rate of natural biodegradation of oil in marine temperate-to-polar zones is probably limited by low temperatures and phosphorus concentrations.

5.3.2.2 Oxygen Supply

CFU formation of organisms from a lake in Antarctica with perpetual high dissolved oxygen (HDO) and that from a lake in Virginia with perpetual saturated atmospheric dissolved oxygen (ADO) were sensitive to HDO toxicity (Mikell, Parker, and Simmons, 1984). The former might have been due to inadequate nutrient supply, while the latter had optimum nutrients available. High oxygen uptake may involve metabolically produced intracellular toxic oxygen by-products, such as peroxide and superoxide. Isolates from the first lake were catalase positive, with inducible superoxide dismutase, and all were pigmented.

Petroleum hydrocarbon degradation markedly occurred in superficial marine sediments (0 to 1 cm) where the oxygen concentration was 8 ppm, whereas, such degradation was slower, but detectable in the system incorporating 2 to 3 ppm. Under anaerobic conditions, no degradation was detected (Bertrand, Esteves, Mulyono, and Mille, 1986).

5.3.2.3 Nutrients

Since physical removal or burning of accidentally spilled oil is seldom feasible, and dispersion or sinking may adversely affect marine life, artificially stimulated biodegradation is being considered as a possible alternative (Atlas and Bartha, 1972b). For this approach to be successful, it is essential that the biodegradation-limiting parameters in seawater should be properly identified.

Oil biodegradation in arctic coastal ponds was found to be nutrient-limited (Atlas and Busdosh, 1976). The addition of an oleophilic nitrogen and phosphorus fertilizer permitted degradation in a previously inactive freshwater pond by increasing the number of oil-degrading microorganisms. It also enhanced the degradation observed in a saltwater pond.

The phosphorous and nitrogen contents of 100 ml unsupplemented seawater would support less than 0.1 mg (dry wt) microbial biomass (Atlas and Bartha, 1972b). This appears to be a major cause for the slow rate of petroleum biodegradation in the sea, especially when vigorous circulation does not occur in the water column underneath the oil slick. Either nitrogen or phosphorous deficiency will tend to produce cells with abnormally high lipid stores and low metabolic activity and may also cause the accumulation of extracellular intermediary metabolites.

An oleophilic nitrogen and phosphorus fertilizer has been developed (Atlas and Bartha, 1973a) and tested for its ability to stimulate petroleum degradation by indigenous organisms in several environments (Atlas and Schofield, 1975; Spain, Milhous, and Bourquin, 1981; Atlas, 1975a). The fertilizer contains paraffinized urea and octyl phosphate. (Optimal C/N and C/P ratios were 10:1 and 100:1, respectively (Atlas and Bartha, 1973a). When

it was tested *in situ* and *in vitro* in near shore areas and several ponds, it stimulated biodegradation of an oil slick by 30 to 40 percent. It did not have any harmful effects on the algae or invertebrate assay organisms.

Iron is also in low concentration in seawater (Atlas, 1977) and is potentially limiting for petroleum biodegradation in nitrogen and phosphorus-supplemented seawater (Dibble and Bartha, 1976). It can be encapsulated or supplied in oleophilic form to meet the nutritional requirements of oil-degrading microorganisms. Additional stimulation was found when oleophilic iron and ferric octoate were added along with nitrogen and phosphorus. Oleophilic iron may be especially beneficial in open oceans, where iron concentrations are low. Use of an oleophilic fertilizer that concentrates the nutrients at the water surface has been shown to increase the degradation of oil slicks (Ward and Lee, 1984) and reduces nutrient loss by diffusion (Atlas and Bartha, 1972d).

In environments without extensive oil pollution history, oleophilic fertilizers may be used to best advantage in combination with microbial inocula (Bartha and Atlas, 1977). Microencapsulation techniques may offer the best solution for a single formulation of organisms and fertilizer.

Another suggestion is to encapsulate a nitrogen-phosphorus fertilizer in a matrix that would allow it to float and be slowly released (Gholson, Guire, and Friede, 1972). A slow-release fertilizer containing paraffin-supported magnesium ammonium phosphate as the active ingredient considerably enhances petroleum biodegradation (Olivieri, Bacchin, Robertiello, Oddo, Degen, and Tonolo, 1976). This mixture is recommended for improving oil biodegradation in seawater (Kator, Miget, and Oppenheimer, 1972).

Assuming that oxygen is always in excess and the contaminating oil has a very large surface exposure to the aqueous phase, the amount of nitrogen required for the destruction of a unit quantity of oil can be calculated. It has been found to be about 4 nmol of nitrogen per ng of oil (Floodgate, 1979). The values for nitrogen demand (analogous to the concept of biochemical oxygen demand) can be converted into volumes of water containing the required amount of nitrogen (Floodgate, 1976). If the nitrogen turnover rate is known for a given body of water, the rates of degradation to be expected can be calculated for summer and winter temperatures. These were found to be 30 g/cubic meter/yr in summer and around 11 g/cubic meter/yr in winter for the Irish Sea. The concentration of elemental nitrogen required to bring about the disappearance of 1 mg of hexadecane by a *Nocardia* sp. is 0.5 mg (Mulkins-Phillips and Stewart, 1974b). This confirms suggestions that the rate of natural biodegradation of oil in marine environments is not limited by the concentrations of nitrogen occurring naturally, but rather by low temperatures and phosphorus concentrations.

Application of fertilizers to freshwater environments may result in undesirable eutrophication and outgrowth of algae (Atlas, 1977). In eutrophic lakes and ponds, addition of nitrogen and phosphorus may not be necessary. In Lake Mendota, which is becoming eutrophified, nitrogen and phosphorus were found to limit rates of oil biodegradation. However, in oligotrophic water bodies and in marine environments, concentrations of usable nitrogen and phosphorus compounds in surface waters are generally too low to support extensive microbial degradation of oil. If nitrogen and phosphorus are added,

they should be in a form that will not allow them to dissipate from the oil-water interface, such as using an encapsulated fertilizer in a matrix that would float and release the compounds slowly.

5.3.3 Alteration of Organic Contaminants

5.3.3.1 Addition of Surfactants

The use of microorganisms as oil dispersants would cause a minimum of environmental damage with a low-toxicity surfactant (Bartha and Atlas, 1977). An Arthrobacter strain, designated as RAG 1, has proved to be a highly efficient emulsifier when growing on crude oil (Reisfeld, Rosenberg, and Gutnik, 1972). The low cell yield, coupled with extensive decrease of benzene-extractable hydrocarbons, suggests the production of degradation intermediates, most likely fatty acids. Fatty acids have been established as biogenic dispersants. In addition, high molecular weight extracellular polymers that are anthrone positive and are precipitated by 95 percent ethanol are produced by several pseudomonads and by Corynebacterium hydrocarbonoclastus (Zajic, Suplisson, and Volesky, 1974). The polymers were effective dispersants also in the absence of the bacteria and acted as flocculants (Knetting and Zajic, 1972).

Several dispersants were tested on marine samples to determine their effects on the degradation of oil (Atlas and Bartha, 1973e). They were found to increase the rate of mineralization, provided the seawater was amended with nitrate and phosphate, but did not increase the extent of degradation. Four dispersants were tested and only one was found that stimulated biodegradation (Mulkins-Phillips and Stewart, 1974c). Two "oil herders" were tested and determined to increase the mineralization rate, but not the extent of degradation (Atlas and Bartha, 1973e).

In another instance, mixed bacterial cultures isolated from foam on the surface of contaminated seawater were found to induce the production of surface-active agents that emulsified the hydrocarbons on which they were growing and improved degradation of these compounds. This ability was not detected with cultures from sea water or sediments.

Within terrestrial ecosystems, such as beach sands, the presence of some emulsifying agents has been found to have a neutral effect on rates of oil biodegradation (Bloom, 1970). Tarry materials occurring on soil or beaches can be physically broken up, e.g., by dicing (Atlas, 1977; Bloom, 1970; Cobet and Guard, 1973). This increases the available surface area for microbial colonization. Breaking up tar globules also increases surface area and, thus, the availability of oxygen, water, and nutrients (Gibbs, 1976).

5.4 TREATMENT TRAINS WITH CHEMICAL AND BIOLOGICAL PROCESSES

No two contamination incidents are exactly alike (Bartha and Atlas, 1977). Consequently, control responses should be flexible and tailored to the situation, rather than follow a rigid pattern. Stimulated biodegradation is not expected to replace all other control measures, but it should rather add further flexibility to integrated control programs.

Treatment trains employing one or more treatment processes may be required for complex waste streams (Lee and Ward, 1986); and bioreclamation can be preceded by, or otherwise used in combination with, other treatments that could reduce toxic concentrations to a tolerable level (Environmental Protection Agency, 1985b). These on-site or in situ treatment techniques could destroy, degrade, or by other means reduce the toxicity of contaminants. Chemical detoxification techniques include injection of neutralizing agents for acid or caustic leachates, addition of oxidizing agents to destroy organics or precipitate inorganic compounds, addition of agents that promote photodegradation or other natural degradation processes, extraction of contaminants, immobilization, or reaction in treatment beds (Lee and Ward, 1986). Some of these processes are discussed in this section. Biological on-site methods, such as treatment of withdrawn groundwater, can be used in conjunction with the in situ practices.

Biological treatment is the least expensive method of organic destruction (Environmental Protection Agency, 1985b). About 99 percent of all organic compounds can be destroyed by biological reactions. When used with other treatment technologies, essentially all the organic contaminants can be removed and destroyed.

Table A.5-11 shows a number of unit operations and the waste types for which they are effective (Ward and Lee, 1984). Table A.5-12 indicates that biological methods can attain a greater level of treatment in groundwater than either stripping or sorption (Knox, Canter, Kincannon, Stover, and Ward, 1968).

5.4.1 Supplementary Processes

Volatile organics, extractable organics, and inorganics (heavy metals) of concern in contaminated groundwater can be treated successfully by two alternative processes (Stover and Kincannon, 1983). 1) One process consists of chemical precipitation to remove metals, steam stripping, and activated carbon adsorption. 2) The alternative consists of combined physical-chemical and biological treatment. Metals treatment in the latter would be a safety measure against possibly higher concentrations than anticipated. It would also be required for removing high iron and manganese concentrations. Combining the unit processes of chemical precipitation, steam stripping, and biological treatment is the most feasible treatment alternative. With method 1), the concentrations of residual organics, measured as TOC, would still be too high.

5.4.1.1 Neutralization

Neutralization involves injecting dilute acids or bases into the groundwater to adjust the pH (Environmental Protection Agency, 1985b). This can serve as a pretreatment prior to in situ biodegradation to optimize the pH range for the microorganisms.

Table A.5-11. Summary of Suitability of Treatment Processes (Ward and Lee, 1984)

Process	Volatile Organics	Nonvolatile Organics	Inorganics
Air stripping	Suitable for most cases	Not suitable	Not suitable
Steam stripping	Effective concentrated technique	Not suitable	Not suitable
Carbon adsorption	Inadequate removal	Effective removal technique	Not suitable
Biological	Effective removal technique	Effective removal technique	Not suitable metals toxic
pH adjustment precipitation	Not applicable	Not applicable	Effective removal technology
Electrodialysis	Not applicable	Not applicable	Inefficient operation/ inadequate removal
Ion exchange	Not applicable	Not applicable	Inappropriate technology-- difficult operation

Table A.5-12. Removal Mechanisms of Toxic Organics from Groundwater (Knox, Canter, Kincannon, Stover, and Ward, 1968)

Compound	Percent Treatment Achieved		
	Stripping	Sorption	Biological
Phenol	--	--	99.9
Aromatics			
Benzene	2.0	--	97.9
Toluene	5.1	0.02	94.9
Ethylbenzene	5.2	0.19	94.6
Halogenated Hydrocarbons			
Methylene Chloride	8.0	--	91.7
Polynuclear Aromatics			
Phenanthrene	--	--	98.2
Naphthalene	--	--	98.6
Other			
Ethyl Acetate	1.0	--	98.8

Adjustment of the pH may be required to make the water less corrosive and suitable for other unit processes (Stover and Kincannon, 1983).

5.4.1.2 Oxidation/Reduction

Oxidation of inorganics in soils is, for all practical purposes, limited to oxidation of arsenic and possibly some lead compounds by use of potassium permanganate. Hydrogen peroxide, ozone, and hypochlorites are the most useful oxidizing agents available. Ozone oxidizes many organic compounds that cannot be easily broken down biologically, including chlorinated hydrocarbons, alcohols, chlorinated aromatics, pesticides, and cyanides (Lee and Ward, 1986).

Chromium can be reduced from the hexavalent state to the trivalent and then precipitated with hydroxide (Lee and Ward, 1986). Reducing agents for chromium include gaseous sulfur dioxide, iron sulfate, waste pickling liquor from metal plating industries, and sodium bisulfite with sulfuric acid commonly used to reduce the pH (Ehrenfeld and Bass, 1984). Levels of less than 1 ppm chromium can be achieved.

Wet-air oxidation involves addition of air at high pressures and temperatures in a form of combustion (Lee and Ward, 1986). A catalyst promotes the oxidation process. Dilute wastes that cannot be treated with incineration can be handled by this process with greater than 99 percent destruction.

5.4.1.3 Precipitation

Precipitation of certain waste components can be accomplished by adding a chemical that reacts with the hazardous consitituent to form a sparingly soluble product or by adding a chemical or changing the temperature to reduce the solubility of the hazardous constituent (Ehrenfeld and Bass, 1984).

Chemical precipitation with carbonate, sulfides, or hydroxides has been used routinely to chemically treat wastewaters containing heavy metals and other inorganics (Knox, Canter, Kincannon, Stover, and Ward, 1984). Sulfides are probably the most effective for precipitating heavy metals; however, sulfide sludges are susceptible to oxidation to sulfate, which may release the metals.

The hydroxide system with lime or sodium hydroxide is widely used but may produce a gelatinous sludge, which is difficult to dewater (Knox, Canter, Kincannon, Stover, and Ward, 1984). Removal of metals by chemical precipitation with lime requires a pH at which a soluble form of the metal is converted to an insoluble form (Stover and Kincannon, 1983).

Soda ash is employed with the carbonate system and may be difficult to control (Knox, Canter, Kincannon, Stover, and Ward, 1984). Alum is another common agent used in chemical precipitation. The effectiveness of these chemical treatments will vary with the nature and concentration of the constituents of the waste stream (Lee and Ward, 1986). A process design for chemical precipitation will have to consider the systems for chemical addition and mixing, the optimal chemical dose, the time required for flocculation and the removal and dispsal of te sludge.

5.4.1.4 Permeable Treatment Beds

Permeable treatment beds can intercept a plume and provide a reactor for either chemical treatment or precipitation (Lee and Ward, 1986). These are essentially excavated trenches placed perpendicular to groundwater flow and filled with an appropriate material to treat the plume as it flows through the material (Environmental Protection Agency, 1985b). Various materials could be used as the fill. Limestone (limestone containing little magnesium carbonate is more effective in removing ions than dolomitic limestone) or crushed shell could be used for neutralizing acidic groundwater and retaining certain metals (such as cadmium, iron, and chromium). Activated carbon could be employed for removing nonpolar organic compounds from contaminated plumes, but not polar organics or heavy metals. Glauconitic green sands have the potential for removing heavy metals, especially copper, mercury, nickel, arsenic, and cadmium. Synthetic ion exchange resins may also be used as fill to remove heavy metals, although they may have a short lifetime, high costs, and be difficult to regenerate. Permeable treatment beds may plug or exhibit channeling, which will reduce their effectiveness. These beds have the potential to reduce the quantities of contaminants present in leachate plumes and are applicable to relatively shallow groundwater tables containing a plume. They have the potential problem of the saturation of bed material, plugging of the bed with precipitates, and short life of the treatment materials.

5.4.1.5 Soil Flushing

Soil flushing is an extraction process to remove organic and inorganic contaminants from contaminated soils (Environmental Protection Agency, 1985b). Water or an aqueous solution is injected into the area of contamination, and the contaminated elutriate is pumped to the surface for removal, recirculation, or on-site treatment and reinjection. During elutriation, sorbed contaminants are mobilized into solution by reason of solubility, formation of an emulsion, or by chemical reaction with the flushing solution.

Solutions with the greatest potential for use in soil flushing would be either water, acids/bases, complexing and chelating agents, surfactants, and certain reducing agents (Environmental Protection Agency, 1985b). Water can be used to flush water-soluble or water-mobile organics and inorganics. Organics amenable to water flushing can be identified according to their soil/water partition coefficient, or estimated using the octanol/water coefficient. Inorganics that can be flushed from soil with water are soluble salts, such as the carbonates of nickel, zinc, and copper. Adjusting the pH with dilute solutions of acids or bases will enhance inorganic solubilization and removal.

Weak acids (e.g., sodium dihydrogen phosphate and acetic acid) or dilute solutions of strong acids (e.g., sulfuric) could be used if the soil contains sufficient alkalinity to neutralize it (Environmental Protection Agency, 1985b). Complexing and chelating agents may also be employed. These can mobilize metals strongly adsorbed to manganese and iron oxides in soils. Surfactants can improve the solvent property of the recharge water, emulsify nonsoluble organics, and enhance removal of hydrophobic organics sorbed onto soil particles. This is a promising *in situ* chemical treatment method.

It would be economically feasible to recycle the elutriate from soil flushing back through the contaminated soil (Environmental Protection Agency,

1985b). Soil flushing methods involving the use of water surfactants appear to be the most feasible and cost-effective chemical treatment for organics. They can use relatively cheap, innocuous treatment reagents, can be used to treat a broad range of waste constituents, and do not result in toxic degradation products. Although a laboratory experiment can be conducted to estimate the number of times the groundwater would have to be turned over or filtered through the contaminated soil to achieve to required level of water quality (Stover and Kincannon, 1983), this process has not proved effective.

5.4.1.6 Carbon Adsorption

Organics can be trapped on activated carbon or on resins by both physical and chemical forces and removed from the groundwater (Nielsen, 1983). The degree of sorption onto the carbon depends upon (Knox, Canter, Kincannon, Stover, and Ward, 1984):

1. The solubility of the compound, insoluble compounds being more likely to be adsorbed

2. The pH of the water, which controls the degree of ionization of the compounds; acids are adsorbed better under acidic conditions and adsorption of amine-containing compounds is favored under alkaline conditions

3. Characteristics of the adsorbent, which is a result of the process used to generate and activate the carbon

4. Properties of the compound; for example, aliphatic compounds are less well adsorbed than aromatics and halogenated compounds

Effluent levels of between 1 and 10 ug/l can be achieved for many organics (Ehrenfeld and Bass, 1984). Partial adsorption of several heavy metals also occurs. Granular or powdered activated carbon has been used. Granular activated carbon is typically employed in reactors, and the powdered carbon is added to the wastewater then either settled or filtered for removal with the sludge.

Carbon adsorption removes low concentrations of organic contaminants or residual organics from other treatment systems. It is the best system for emergency response. Activated carbon systems can be batch, column, or fluidized-bed reactors (Lee and Ward, 1986). See Appendix F for more information on this process.

5.4.1.7 Air and Steam Stripping

Under some circumstances, soil containing volatile hydrocarbons or solvents can be decontaminated by air stripping (Niaki, Pollock, Medlin, Shealy, and Broscious, Draft). In this process, air is injected into the soil through a series of extraction wells. It is blown into the injection wells and pulled out of the extraction wells. As it flows through the soil, volatile materials are stripped off into the air stream. The organics are removed from the air in a vapor phase carbon adsorption system or by fume incineration. The success of in situ air stripping depends upon relatively unrestricted and

uniform flow of air through the soil. Clay soils, packed soils, or soils with a high water table are not good candidates for in situ air stripping.

Volatile compounds can also be removed from withdrawn groundwater by air or steam stripping (Nielsen, 1983). Various configurations of equipment can be used in air stripping, including diffused aeration, countercurrent packed columns, cross-flow towers and coke tray aerators with countercurrent packed columns. The latter is probably the most useful for decontamination of groundwater, since the countercurrent packed columns provide the most interfacial liquid area, high air-to-water volumes, and can be easily connected to vapor recovery equipment (Knox, Canter, Kincannon, Stover, and Ward, 1984). In many applications of air strippers, the volatile compounds are transferred from the water to the air where the vapors can then be collected and treated by incineration or adsorption to carbon (Nielsen, 1983). Increased temperatures can improve the removal efficiency of the stripping process for some compounds, such as aldehydes and alcohols (Law Engineering Testing Company, 1982).

Steam stripping has the same effects as elevated temperature air stripping (Knox, Canter, Kincannon, Stover, and Ward, 1984). Costs for air stripping have been estimated to be between 9 and 90 cents per 1000 gal of water treated for removal of 90 percent of trichloroethylene.

A technique similar to air stripping for the removal of some compounds from contaminated groundwater is that of dissolved air flotation, in which suspended fine particles or globules of oil and grease are floated to the surface by the action of pressurized air and then removed by skimming (Ehrenfeld and Bass, 1984). This technique has been used to remove up to 90 percent of the total suspended solids or oil and grease in wastewater containing 900 ppm of these substances.

5.4.1.8 Reverse Osmosis

This process may be used to concentrate inorganics and some high molecular weight organics from waste streams (Lee and Ward, 1986). It passes the contaminated water through a semipermiable membrane at high pressure. The clean water leaves behind the concentrated wastes and any particulates. Pretreatment of the waste stream is likely to be required to achieve a constant influent composition--pH is particularly important--to kill any organisms that might form a biological film that would reduce permeability, to remove suspended solids, and to remove chlorine, which might affect the membrane.

5.4.1.9 Mobilization/Immobilization

Surfactants may be added during soil washing to mobilize the contaminants (Ellis, Payne, Tafuri, and Freestone, 1984). A four percent solution of two nonionic surfactants removed greater than 90 percent of polychlorinated biphenyls and a high boiling distillation fraction of crude oil from test soil columns with 10 pore volume washes.

Contaminants can be immobilized by precipitation or encapsulation in an insoluble matrix (Pye, Patrick, and Quarles, 1983). A spill of acrylate monomer was treated with catalyst and activator in order to produce a solidified polymer, thereby, immobilizing an estimated 85 to 90 percent of the liquid monomer (Knox, Canter, Kincannon, Stover, and Ward, 1984).

5.4.2 Examples of the Use of Treatment Trains

* In a study of a site polluted by hydrocarbons, chlorinated hydrocarbons, and organo-chloride pesticides, it was found that no single technology could remove or destroy all of the contaminants (Rickabaugh, Clement, Martin, and Sunderhaus, 1986). However, when microbial degradation, surfactant scrubbing, photolysis, and reverse osmosis were combined, nearly total destruction of these compounds could be attained on-site.

* This approach was also employed at a site where 130,000 gal of several organics had been spilled (Lee and Ward, 1985). Treatment of the site was by clarification, adsorption onto granular activated carbon, air stripping, then reinjection. After levels of the contaminants had fallen below 1,000 mg/l, a biodegradation program employing facultative hydrocarbon-degrading bacteria, nutrients, and oxygen was begun. Biodegradation by both the indigenous microbes and the added organisms reduced the levels of the contaminants in soil cores from 25,000 to 2,000 mg/l within two months. The monitoring wells showed no levels above 1 mg/l at the end of the program.

* Another example involving the use of multiple treatment processes was a bench-scale study on a site in Muskegon, MI, contaminated by several priority pollutants and at least 70 other organics at levels in the hundreds of ppm (Lee and Ward, 1985). Acclimation of an activated sludge culture to the contaminated groundwater was unsuccessful, and a commercial microbial culture was ineffective at degrading the contaminants. However, coupling an activated sludge process to granular activated carbon treatment proved beneficial, as the organisms were able to degrade the organics that passed through the carbon system. This treatment train was able to remove up to 95 percent of the total organic carbon in the wastewater, as long as the activated carbon continued to function properly.

* The release of phenol and chlorinated derivates in the soil in the Midwest was treated by installing a recovery system and using activated carbon filters on the groundwater (Walton and Dobbs, 1980). Surface waters were contained in a pond. Mutant bacteria were injected into the pond and into the contaminated soil. After an incubation and adaptation period, the phenol was completely degraded in 40 days, while the ortho-chlorophenol was reduced from 120 to 30 ppm.

* Additional examples of the use of treatment trains in actual field cases can be found in Appendix E.

5.5 BIODEGRADATION IMPLEMENTATION PLAN

The basic steps in an *in situ* bioreclamation process are (Lee and Ward, 1985; Buckingham, 1981):

Free product recovery
Site investigation
Well design
Well installation
Microbial degradation optimization study
System design
Operation
Groundwater sampling
Sampling techniques
Groundwater testing
Monitoring
Oxygen management of groundwater
Nutrient management of groundwater

5.5.1 Free Product Recovery

Free product or the source of the contamination should be removed before a treatment program is initiated (Nielsen, 1983). Physical recovery methods are used to recover as much of the free product as possible (Lee and Ward, 1985).

The design of a free product recovery system is greatly affected by the geohydrologic characteristics of the site (Niaki, Pollock, Medlin, Shealy, and Broscious, Draft). Groundwater flow, lithologic permeability, and gravity have the greatest effect on hydrocarbon migration in subsurface environments (Van Dam, 1967). Use of equations can allow estimation of the actual thickness of the hydrocarbon saturation zone, based upon measured hydrocarbon thickness in monitor wells and lithology. The hydrocarbon layer in a monitor well may be one to ten times as thick as the actual hydrocarbon saturated zone in the surrounding soil. Geologic bedding dip, as well as structural features, may also control hydrocarbon movement (Osgood, 1974).

Free product can be recovered by several techniques (Canter and Knox, 1984). Pumping operations to collect the floating lens include multiple-level pumps with hydrocarbon detectors and single-level skimming pumps (Jeter, 1985). A single-pump system utilizing one or more wells requires minimal equipment and drilling costs, but produces a mixture of product and water that must be separated (Canter and Knox, 1984). A two-pump, two-well system utilizes one well to produce a water table gradient that allows the second well to recover the floating product. Another type of system utilizes a single well with two pumps--a lower pump to produce a gradient and an upper one to collect the free product. Well systems represent a proven method for control of many hazardous waste contamination problems. However, they do have high maintenance and operation costs; limited application to fine soils; removal of clean water along with contaminated water, which increases the costs of treatment; and long operation times, especially to remove sorbed contaminants.

There are also several other technologies for bulk gasoline recovery (Jeter, 1985). Interceptor trenches dug below the water table can be used to collect floating hydrocarbon layers. Dissolved product has been treated by

air stripping and granular activated carbon (GAC). However, air stripping may discharge these contaminants into the atmosphere. A significant proportion of the product from a spill or leak will remain in the unsaturated soil zone and be unavailable for free product recovery under existing conditions. This material can then be subjected to biological treatment (Hoag and Marley, 1986).

Local and Federal agencies do not accept bulk fuel removal as a complete restoration. If practical, it may be wise to continue to pump contaminated wells to contain the contaminants (Raymond, Jamison, and Hudson, 1976).

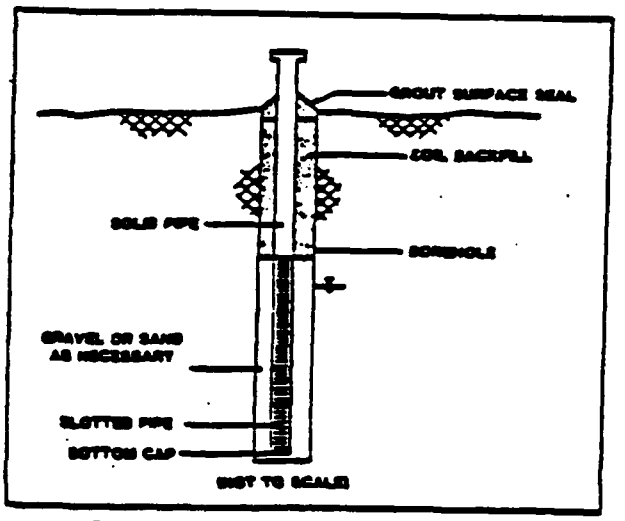
5.5.2 Site Investigation

Implementation of the most effective and cost-beneficial procedure for treating a hazardous waste requires adequate site assessment of both the saturated zone and the vadose zone (Buckingham, 1981). A thorough understanding of the hydrogeology and extent of contamination of the site must be obtained and used to design the treatment system (Lee and Ward, 1986; Knox, Canter, Kincannon, Stover, and Ward, 1968). This is usually accomplished by installation of a network of monitoring wells and characterization of the aquifer through pump tests, analysis of soil properties, and other methods (Roberts, Koff, and Karr, 1988). The stratigraphy beneath the site and a description of the soils and bedrock should be detailed, including the presence and extent of inhomogeneities in the soil (Lee and Ward, 1986; Quince and Gardner, 1982). The hydrogeologic data needed are formation porosity, hydraulic gradient, depths of both the aquifer and the contaminated zone, permeability, groundwater velocity and direction, specific yield of the aquifer, and recharge/discharge information. Knowledge of other environmental parameters, such as precipitation, temperature (Buckingham, 1981), soil moisture, pH, oxidation-reduction potential, and oxygen, nutrient, and organic matter content are necessary. The source, quantity, and nature of the spilled material must also be assessed (Knox, Canter, Kincannon, Stover, and Ward, 1968).

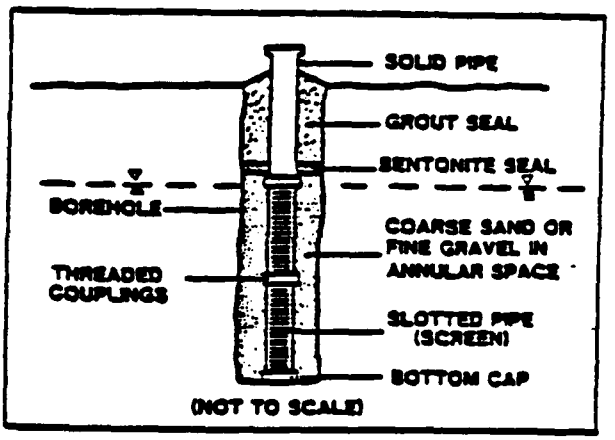
5.5.3 Well Design

A well is an excavated shaft in the ground that may be lined or cased with some material to keep it from caving in, often with plastic or metal pipe (Bitton and Gerba, 1985). When determining specific well structure, all inflows of water, except through the zone of interest, should be sealed out. Three well types are diagrammed in Figure A.5-4 (Absalon and Starr, 1980). Type I well is for use in preliminary studies, prior to planning and implementation of additional studies. A grout seal prevents groundwater contamination by surface waters, but the well has obvious limitation in that soil water can percolate down the borehole to the groundwater level. This well does not meet the Resource Conservation and Recovery Act's requirements for groundwater quality monitoring well construction. Type II well has a combination bentonite and grout seal around that section of the well above the water table. It meets EPA guidelines for monitoring well construction. Type III well is grouted and sealed from the ground surface to the top of the zone of interest. It is intended for difficult drilling applications and long-term monitoring.

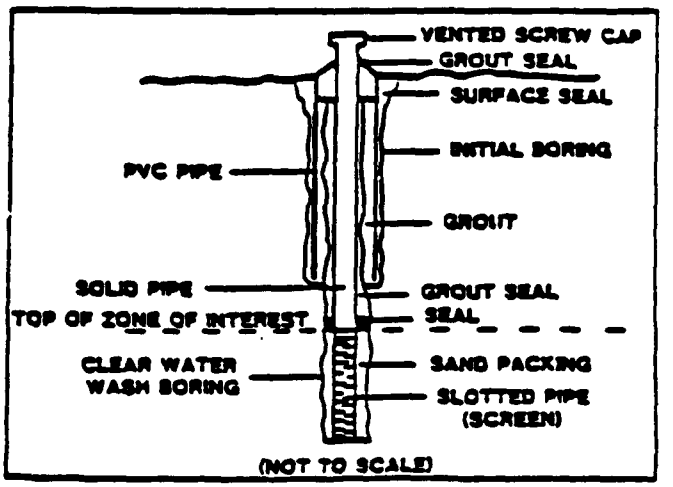
Materials used in well construction typically depend upon the mechanical strength required. Shallow wells (less than 50 ft) are normally constructed of



Schematic of Type I Well



Schematic of Type II Well



Schematic of Type III Well

Figure A.5-4. Three Types of Wells (Absalon and Starr, 1980)

schedule 40 polyvinylchloride (PVC) plastic, and deeper wells are constructed of schedule 80 PVC. PVC is relatively inert, although certain contamination problems may be associated with its use. Organic constituents of groundwater are adsorbed on PVC casing. The casing may also contribute low levels of organic contaminants to groundwater samples, such as phthalic acid esters used as plasticizers in PVC manufacture, and solvents from cements used to join lengths of PVC tubing (Dunlap, McNabb, Scalf, and Cosby, 1977). Teflon is currently being investigated as an alternate well construction material. However, it has two major drawbacks--high cost and relative softness (Absalon and Starr, 1980). Type III well is constructed of PVC or stainless steel above the water table and PVC or teflon below the water table where the casing is directly in contact with groundwater. If the level of contamination is high, it is probable that the effects of PVC on the water sample will be insignificant.

5.5.4 Well Installation

Installation of a network of monitoring wells and characterization of the aquifer through pump tests and analysis of soil properties will provide understanding of the hydrogeology of the site to plan the installation of an interceptor system. (Nielsen, 1983).

Consideration should be given to the use of extensive monitoring well networks to more adequately characterize the spatial and temporal variability that has been observed (Plumb, 1985). In addition, a substantial number of substances (including several organic priority pollutants) have been detected at a higher frequency or higher concentration at upgradient wells, which suggests that the direction of chemical migration may be influenced by factors other than groundwater flow. Therefore, it should not be assumed that background conditions are pristine and that upgradient monitoring can be minimized. There should also be a number of wells placed at upgradient locations.

The composition of well water will generally be influenced by well installation methods (Environmental Protection Agency, 1976). For example, care should be taken in the selection of a water supply, if the well is installed by the wash boring method. This technique introduces large quantities of surface waters into the groundwater, which could yield samples that are unrepresentative of subsurface conditions (Absalon and Starr, 1980). Drilling fluids may alter the subsurface chemical and microbiological environment in well vicinities (Dunlap, McNabb, Scalf, and Cosby, 1977). Drilling mud can inhibit groundwater flow by closing interstitial granular spaces in aquifers and reducing aquifer permeability (Absalon and Starr, 1980).

Several effective well construction methods, such as the air rotary method, appear to have little or no effect on groundwater quality. Augers are an efficient technique for drilling shallow wells (40 to 50 ft), in reasonably compact alluvial materials (Dunlap, McNabb, Scalf, and Cosby, 1977). These techniques should be used where they are physically and economically feasible, in order to minimize groundwater contamination.

With any well construction method, it is extremely important to clean all drilling equipment before the monitoring well is installed (Absalon and Starr, 1980). This prevents cross contamination of wells by drilling tools. It has

been recommended that all casing materials be prepared before installation in a borehole (Dunlap, McNabb, Scalf, and Cosby, 1977). This involves a wash with soap and water, a water rinsing, a solvent rinsing, and a final rinsing with organic-free water.

Cation exchange capacity (CEC) is the sum total of exchangeable cations that a soil can adsorb (Brady, 1974). Finer textured soils tend to have higher CEC values than sandy soils. Cations of various elements are present in soil solution and are transported to groundwater. Bentonite, a sealer used in well construction, is composed predominantly of clay. Therefore, it has a high CEC, which may change the concentration of cations in the groundwater, thus, altering its chemical composition.

5.5.5 Microbial Degradation Optimization Study

The site must be investigated for the presence or absence of microorganisms (e.g., aerobic bacteria, anaerobic bacteria, actinomycetes, fungi, and algae) (Sims and Bass, 1984). A laboratory study must be conducted to determine whether the native microbial population can degrade the components of the spill (Raymond, 1978). If it cannot, the biodegradation approach should either be abandoned or additional studies conducted to determine what microorganisms or combination of organisms should be added for the particular contaminants involved.

Laboratory studies must also characterize the waste. The solubility of the contaminant may have to be increased by adding emulsifiers to allow microbial action on the compound (Knox, Canter, Kincannon, Stover, and Ward, 1968). The C:N:P ratio must be determined. If this ratio is not optimal, bench-scale experiments should be performed to ascertain whether adjusting the ratio will increase the numbers of organisms and the rate of biodegradation. The optimum oxygen, nutrient, and temperature requirements of the indigenous microbial population (or added organisms) must be found for degradation of the specific site contaminants (Lee and Ward, 1986; Knox, Canter, Kincannon, Stover, and WArd, 1968). The study determines what combination of nutrients will give the maximal cell growth on gasoline, for instance, in 96 hr under aerobic conditions at the ambient temperature of the groundwater (Lee and Ward, 1985). Nutrient formulations may differ among sites. The form of the nutrient added also varies, but will usually contain sources of nitrogen, phosphorus, magnesium, carbonate, calcium, manganesé, sulfate, sodium, and iron. A laboratory investigation of the kinetics of biodegradation for the organisms should also be performed (Raymond, 1974). Appendix E provides more information on what should be included in the laboratory studies.

5.5.6 System Design

The system for nutrient and oxygen addition and circulation through the aquifer will have to be designed and built, and should be done under the direction of an experienced groundwater geologist (Lee and Ward, 1986). This system usually consists of injection and production wells to control flow, and equipment to add and mix the nutrient solution (Raymond, 1978). Reinjecting pumped groundwater back into the system will recirculate any unused nutrients, thus, avoiding the problem of disposal of this water, if this approach is permissible in the state in which the contaminant incident has occurred (Lee and Ward, 1985). Creation of a closed loop of recovery, treatment, and

recharge flushes the contaminants out of the soil rapidly and establishes hydrodynamic control separating the contaminated zone from the rest of the aquifer (Knox, Canter, Kincannon, Stover, and Ward, 1968). Another benefit of the closed loop is that acclimated bacteria can be added to the aquifer via the extracted groundwater and can act in situ to degrade the contaminants. The recharge water can be adjusted to provide optimal conditions for the growth of the added bacteria and the indigenous population, which may also act on the contaminants.

5.5.7 Operation

Once the system is constructed, nutrient and oxygen addition can begin (Raymond, Jamison, Hudson, Mitchell, and Farmer, 1978). Nutrients can be added by batch or by continuous feed; continuous feed supplies a more constant nutrient source, but requires more labor and equipment to implement. Large quantities of nutrients may be required. At one site, 16.65 tons of chemicals were added, and at another, a total of 87 tons of food-grade quality chemicals were purchased. Oxygen can be supplied to the aquifer by sparging air into wells.

5.5.8 Groundwater Sampling

Groundwater is obtained from a well by some sort of device to bring water to the surface (Davis, 1967).

There are problems associated with collecting subsurface samples. Subsurface organisms are essentially restricted to the spaces between soil particles (Davis, 1967). These organisms are either free floating in the interstitial water or are attached to the solid phase (Marxen, 1981). Samples of subsurface solids are often taken at only one point and cannot be repeated (Davis, 1967). The microorganisms in water samples from wells may be transient and may not be representative, quantitatively or qualitatively, of the indigenous microbial populations existing in the formation being sampled. However, properly collected well samples allow the investigator to determine the concentration of microorganisms and chemical constituents in the water at a particular point at a given time and over an extended period of time. This will provide useful information about processes occurring in the subsurface. However, the proper collection of subsurface water for microbiological analyses is extremely difficult.

It is hard to collect a representative sample, and it is extremely difficult to collect an uncontaminated one. The best way to study microbial activity in deep formations is to take core samples during drilling. An uncontaminated sample can be best obtained by subsampling a core sample. The outer, 1 to 2 cm of the contaminated portion of the samples are removed, leaving the inner, untouched core for bacterial analysis (Gilmore, 1959). Most coring procedures may prove to be too expensive and time-consuming in a field investigation (Wilson, McNabb, Wilson, and Noonan, 1983; Wilson, Noonan, and McNabb, 1983).

It is assumed that free-floating organisms travel with the groundwater (Davis, 1967). Therefore, wells must be sited and screened to a depth that will intercept a particular flow path. Wells upsite can supply data on water quality for comparison. The depth of screening is important in interpreting

data. Drilling fluids often contain various additives and may be a source of bacterial contamination (McNabb and Mallard, 1984) and should not be used when taking core samples (Scalf, McNabb, Dunlap, Cosby, and Fryberger, 1981). To minimize loss of a sample composed of loose material, pouring liquid nitrogen down the pipe will freeze the material (Danielpol, 1982).

Because water in the unsaturated zone is under pressures less than atmospheric, the water will not move into wells that are exposed to atmospheric pressure (Davis, 1967). Specially designed gravity lysimeters (Prill, Oaksford, and Potorti, 1979) have been used successfully to recover bacteria from the unsaturated zone (Vaughn, Landry, Beckwith, and Thomas, 1981z).

The rate of groundwater movement is generally slow, usually measured in feet/day or feet/year. Environmental conditions are relatively constant (Dunlap and McNabb, 1973). Temperatures in the earth's crust fluctuate only in the topmost layer of about 10 m (33 ft), which may be affected by seasonal temperature variations (Kuznetsov, Ivanov, and Lyalikova, 1963). Therefore, frequent sampling would not be necessary. It may also take months for groundwater to move from a source of contamination to the nearest downgradient well (Davis, 1967).

5.5.8.1 Sampling Techniques

Wells should be pumped or bailed before sampling is initiated, in order to ensure that water samples are representative of the groundwater near the well (Buckingham, 19818). There are no universal rules for the quantity of well water to be extracted. Wells installed through use of drilling fluids generally require extraction of large quantities of water, since they alter groundwater quality more substantially than wells installed with augers or air rotary devices. As a general rule, sampled water should not be turbid (Absalon and Starr, 1980).

Groundwater samples can be retrieved by pumping or bailing techniques (Environmental Protection Agency, 1976). Pumping methods, specifically the use of portable submersible pumps, can be cost-efficient, but they often release compressed air into the well. Reduced pressure pumping systems can strip samples of volatile constituents. In addition, pressure changes in pumped samples can cause changes in oxidation state, pH, and temperature of the sample (Absalon and Starr, 1980). These changes affect the sample's chemical constituents.

A barcad is a porous, hollow alumina cylinder that extracts water and conducts it to the surface through a small diameter rise tube (Gardner and Ayres, 1980). The sampler is activated from the surface by a valve inside the cylinder. Although the barcad uses compressed, inert gas, this technique minimizes agitation of the water sample and is believed to preserve volatile organic compounds better than most conventional methods.

Manual sampling techniques are useful under a variety of circumstances; i.e., when sampling is infrequent, biological or sediment samples are required, hazardous material spills (or other special incidents) are being investigated, or concentrations of substances are relatively constant (Shelley, 1977).

There are several manual sampling techniques. One method uses a small weight attached on the end of a sash cord (Raymond, Jamison, and Hudson, 1976). Properly cleaned and sterilized 6-oz glass bottles are fastened near the weight with half-hitch knots around the body and neck of the bottles. A cork is secured just above the bottle opening. To sample the groundwater, the cork is forced into the bottle, and the bottle is lowered to the desired depth. A sharp jerk of the cord removes the cork in order to permit filling of the bottle. Organic substance tends to cling to sample containers. Therefore, glass bottles are appropriate samplers because they are inert, relative to organic material, and they can withstand a rigorous cleaning procedure (Environmental Protection Agency, 1976).

The teflon bailer, diagrammed in Figure A.5-5, is another manual sampler (Dunlap, McNabb, Scalf, and Cosby, 1977). It is 18 to 36 inches long, constructed of heavy wall teflon, and is plugged at the bottom end with a short length of teflon rod. Water enters the bailer through a 5/16-inch hole drilled through the end plug, as the bailer is lowered into the well. It is prevented from draining out by a 3/4-inch diameter glass marble, which fits into a conical seat machined into the top of the plug. The plug fits snugly inside the tube, making up the body of the bailer. Thus, no adhesives, which could contaminate sampled water, are necessary to hold it in place. Bailers are sterilized by autoclaving before they are used, to minimize well contamination. The bailer is raised and lowered with the use of a cable. Samples are poured from the bailer into clean serum bottles of appropriate size (125 ml). Caution is taken to avoid turbulence, which might result in loss of volatile organics or excessive oxygenation of samples. Serum bottles are topped off to avoid including gas spaces.

Preservation techniques retard chemical and biological changes after a sample is taken (Environmental Protection Agency, 1976). Bacteria are inhibited by refrigeration and acidic conditions (created by the addition of sulfuric acid), and chemical reaction rates are retarded by refrigeration (Environmental Protection Agency, 1974).

5.5.9 Monitoring

Contaminated groundwater must be analyzed not only for the extent of contamination, but also for its oxygen and nutrient levels. A system of monitoring wells is usually established for such purposes. Representative groundwater samples can be properly acquired through well design and construction techniques.

Regular monitoring of the levels of organics and inorganics in the effluent and groundwater is necessary to judge the effectiveness of the treatment and to ensure proper distribution of the nutrients. The response of the bacterial population to nutrient addition should also be monitored. The highest counts of up to 10^7 organisms/ml (Lee and Ward, 1985) will generally be in the areas of greatest contamination, with a ten- to thousandfold increase in the numbers of gasoline-utilizing organisms possible after nutrient addition.

Monitoring a number of parameters is necessary to determine process performance (Environmental Protection Agency, 1985b). Monitoring of groundwater can be performed at the injection and extraction wells, as well as at monitoring wells. On-site wells will monitor process performance, and off-

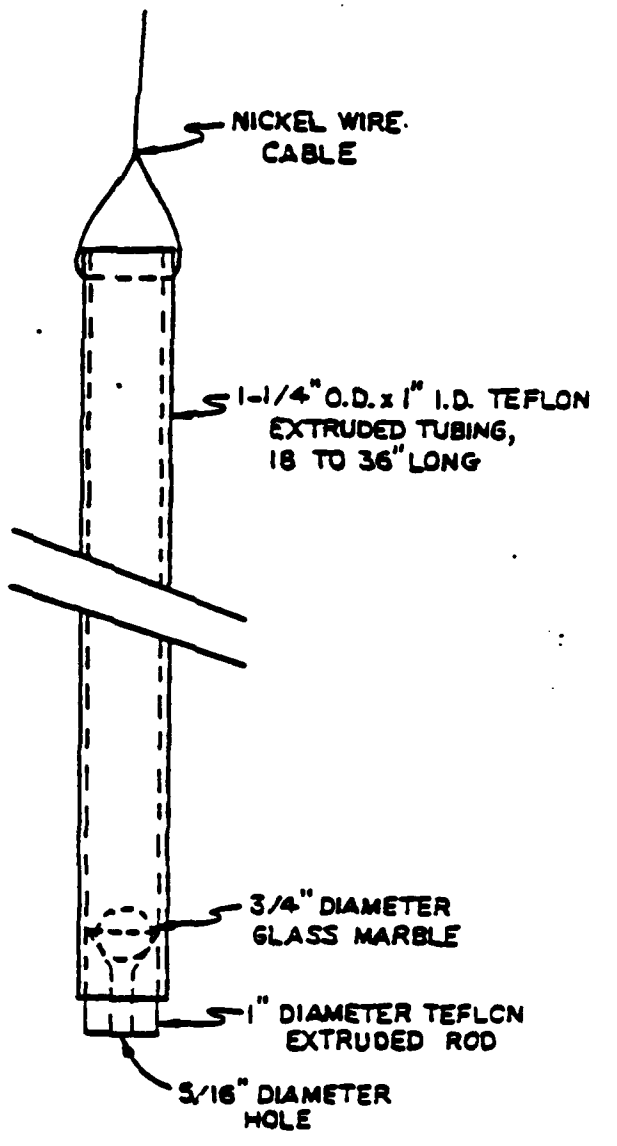


Figure A.5-5. Diagram of a Teflon Bailer (Dunlap, McNabb, Scalf, and Cosby, 1977)

site wells will monitor for pollutant migration, as well as provide background information on changes in subsurface conditions due to seasonal fluctuation. Table A.5-13 lists parameters that should be monitored and suggests methods for monitoring them.

If a monitoring well is not screened properly, its intake might miss the contaminant plume altogether, since groundwater flow and contaminant transport can be extremely stratified (Josephson, 1983a). The plume's thickness might not be as great as that of the aquifer itself. Plume identification involves four dimensions. The monitoring scheme must consider plume spread in two horizontal and one vertical direction over time. This knowledge will help ensure that less uncontaminated water is incidentally pumped with the contaminated for treatment.

5.5.10 Groundwater Testing

The groundwater samples collected from the monitoring can be analyzed on-site or shipped to water testing laboratories for analysis after they have been properly preserved (Buckingham, 1981). Laboratories are available through Federal, state, and municipal governments, as well as through universities and private companies.

This section outlines three specific groundwater tests for oxygen, nitrogen, and phosphorus. The U.S. EPA sampling requirements, preservation techniques, and allowable holding times are summarized in Table A.5-14 (Environmental Protection Agency, 1974).

a. Dissolved oxygen (DO)

Dissolved oxygen can be determined by two methods (Rand, 1975). The Winkler or iodometric method is the most precise and reliable titrimetric procedure for dissolved oxygen analysis. However, organic compounds and oxidizing or reducing agents can interfere with this technique. Therefore, certain modified methods have been developed to minimize these interferences.

The iodometric method is not ideally suited for field testing, continuous monitoring, or for DO determination *in situ*. However, electrodes provide an alternate testing method for such conditions.

Sensing elements of membrane-covered electrode systems are protected by an oxygen-permeable plastic membrane. This serves as a diffusion barrier against impurities that can interfere with results. They are, therefore, especially suited for use in polluted waters where iodometric methods, and even their modifications, are subject to serious errors caused by interferences. Electrode meters are available commercially and yield an accuracy of +-0.1 mg/l DO and a precision of +-0.05 mg/l DO.

b. Biochemical Oxygen Demand (BOD)

A test for biochemical oxygen demand measures the dissolved oxygen consumed by microbial life while assimilating and oxidizing the organic matter present (Environmental Protection Agency, 1976). Analysis involves a five-day incubation period. The reduction of dissolved oxygen during that period yields a measure of BOD.

Table A.5-13. Recommended Parameters to Monitor (Environmental Protection Agency, 1985b)

Parameter	Location of Analysis	Media	Analytical Method
Total organic carbon (TOC)	Laboratory	Groundwater	TOC analyzer
Priority pollutant analysis or analysis of specific organics	Laboratory	Soil and groundwater	GC/MS[a]
Microbiology-cell counts	Laboratory	Soil and groundwater	Direct counts. Plate counts on groundwater media or enriched media
	Field	Groundwater	Plate counts with portable water test kits (e.g., Soil Test Inc., Evanston, IL)
Temperature conductivity dissolved oxygen (DO) pH	Field	Groundwater	In situ water quality monitoring instrument or prepackaged chemicals, field test kits
Alkalinity acidity, M&P chloride hardness (total) NH_3-N NO_3-N PO_4, all forms SO_4 TDS (total dissolved solids)	Field	Groundwater	Prepackaged chemicals/ field test kits; water analyzer photometer (Soil Test, Inc.; Lamotte Chemical, Chestertown, MD)
Heavy metals (if present)	Field Laboratory	Groundwater Soil and groundwater	Prepackaged chemicals/ test kits; GC/MS; AAS[b]

Table A.5-13. Recommended Parameters to Monitor (Environmental Protection Agency, 1985b) (Continued)

Parameter	Location of Analysis	Media	Analytical Method
Hydrogen peroxide	Field	Groundwater	Prepackaged chemicals for H_2O_2, test strips available, Titanium sulfate titration and spectrophotometer analysis for greater accuracy

[aa]GS/MS = gas chromatography/mass spectrometry
[b]AAS = atomic absorption spectrometry

Table A.5-14. U.S. EPA Sampling Requirements, Preservation Techniques, and Allowable Holding Times (Environmental Protection Agency, 1974)

Measurement	Volume Reqd (ml)	Container	Preservative	Holding Time
Dissolved oxygen (DO)				
Probe (in situ) DO concentration determined on-site				no holding
Winkler (in lab)	300	glass	[a]	4-8 hr
Biochemical oxygen demand (BOD)	1000	glass or plastic	cool, 4°C	6 hr[b]
Chemical oxygen demand (COD)	50	glass or plastic	H_2SO_4 to pH 2	7 days
Nitrogen				
Ammonia	400	glass or plastic	cool, 4°C H_2SO_4 to pH 2	24 hr
Nitrate	100	glass or plastic	cool, 4°C H_2SO_4	24 hr
Nitrite	50	glass or plastic	cool, 4°C	24 hr
Kjeldahl total	500	glass or plastic	cool, 4°C H_2SO_4 to pH 2	7 days
[c]Phosphorus				
Orthophosphate, dissolved	50	glass or plastic	filter on-site cool, 4°C	24 hr
hydrolyzable	50	glass or plastic	cool, 4°C H_2SO_4	24 hr
total	50	glass or plastic	cool, 4°C	7 days

Table A.5-14. U.S. EPA Sampling Requirements, Preservation Techniques, and Allowable Holding Times (Environmental Protection Agency, 1974) (Continued)

Measurement	Volume Reqd (ml)	Container	Preservative	Holding Time
Total, dissolved	50	glass or plastic	filter on-site cool, 4 ^{o}C	24 hr

a = To preserve the sample for Winkler determination, add 0.7 ml concentrated H_2SO_4 and 1 ml sodium azide solution to a 300 ml DO sample (Rand, et al., 1975).

b = If samples cannot be returned to the laboratory in less than 6 hr, and holding time exceeds this limit, the final reported data should indicate the actual holding time.

c = Do not store samples containing low concentrations of phosphorus in plastic bottles because phosphate may be adsorbed onto the walls of the bottles (Rand, et al., 1975).

Samples for BOD analysis may undergo significant decay during handling and storage (Thibault and Elliot, 1980). A portion of the demand may be satisfied, if the sample is stored for several days before the test is initiated. This results in a lower BOD value than is actually present in groundwater.

c. Chemical Oxygen Demand (COD)

A test for chemical oxygen demand measures the oxygen equivalent of that fraction of organic matter in a sample that may undergo oxidation by a strong organic or inorganic chemical oxidant (Rand, et al., 1975). COD is determined by the dichromate reflux method. The oxidant in this test has advantages over other oxidants in applicability to a variety of samples, oxidizability, and ease of manipulation.

d. Nitrogen

Nitrate, nitrite, ammonia, and organic nitrogen are forms of nitrogen that are of interest in waters and wastewaters. They are interconvertible and are components of the nitrogen cycle.

Ammonia is naturally present both in surface and groundwater. It may be produced by the reduction of nitrate under anaerobic conditions. Therefore, a high ammonia concentration can indicate anaerobic conditions in groundwater.

Three methods are available to test for ammonia. The Nessler method is sensitive up to 20 ug/l ammonia nitrogen under prime conditions, and it is suitable for testing ammonia nitrogen concentrations of up to 5 mg/l. Interfering substances may be removed primarily by distillation. The phenate method has a sensitivity of 10 ug/l ammonia nitrogen, and it can test for ammonia nitrogen concentrations of up to 500 mg/l. Preliminary distillation is required, if the alkalinity exceeds 500 mg/l or if color or turbidity is present, if the sample has been preserved with acid, or if the ammonia nitrogen concentration is greater than 5 mg/l.

Nitrate is generally present in low concentration in surface waters, but it may attain high levels in some groundwater. It is a required nutrient for many photosynthetic autotrophs and has been recognized as the growth-limiting factor in some cases. In high concentrations, however, it contributes to the illness known as infant methemoglobinemia. Therefore, a limit of 10 mg/l nitrate has been imposed on drinking water, in attempts to prevent this disorder. A number of colorimetric procedures are available for determining nitrate levels.

Nitrate is difficult to determine because of the relatively complicated procedures required, the high probability that interfering substances will be present, and the limited concentration ranges of the various methods. Two screening techniques are available to initially determine the approximate range of nitrate in the sample. A method suitable for the concentration range of the sample is subsequently selected.

There are six methods available to test for nitrate nitrogen concentration. The ultraviolet spectrophotometric method is used for screening only those samples with low organic matter content. The cadmium reduction method reduces nitrate almost quantitatively to nitrite, when a sample is run

through a column containing amalgamated cadmium filings. The brucine method involves a reaction between nitrate and brucine that produces a yellow color. Final nitrate concentration is subsequently determined by colorimetry. The chromotropic acid method is recommended for the concentration range 0.1 to 5 mg NO_3-N/l. In this method, nitrate reacts with chromotropic acid to form a yellow reaction product exhibiting maximum absorbance at 410 nm. The absorbance is read on a spectrophotometer to determine nitrate concentration. Devarda's alloy reduction technique is recommended for those samples in which the nitrate-nitrogen concentration is greater than 2 mg/l. In this method, nitrate and nitrite are reduced to ammonia under hot alkaline conditions in the presence of the reducing agent, Devarda's alloy. For field use, the nitrate electrode method is the simplest and most practical technique. It is used for screening water and wastewater samples.

Nitrite is an intermediate in the nitrification process. Its concentration is determined by the diazotization method in the nitrite-nitrogen range of 1 to 25 ug/l nitrogen. Photometric measurements can be made in the range of 5 to 50 ug/l.

Organic nitrogen and ammonia can be determined together and have been referred to as "total nitrogen" or "kjeldahl nitrogen," a term that reflects the method used in their determination. The "organic nitrogen" can be obtained by the difference of the individual kjeldahl nitrogen and ammonia nitrogen determinations.

e. Phosphorus

Phosphorus occurs principally as phosphate compounds in natural waters and wastewaters (Rand, et al., 1975). These compounds are usually categorized into orthophosphates, condensed phosphates, and organically bound phosphates. These may occur in the soluble form, or they may be incorporated into particles of detritus, or into the cells of organisms. Phosphorus fertilizer is generally applied as orthophosphate. Phosphorus is necessary for the growth of organisms, and it can be the nutrient that limits the productivity of a body of water.

Phosphate analyses comprise two general steps: 1) conversion of the phosphorus form of interest to soluble orthophosphate and 2) colorimetric determination of soluble orthophosphate. For analytical purposes, phosphate has been classified into four chemical types: total, ortho, acid-hydrolyzable, and organic. Each of these types can be tested in three different physical states: total, filterable (dissolved), and nonfilterable (particulate).

Phosphorus may exist in suspension and may combine with organic matter. As a result, a digestion method to determine total phosphorus must be effective both in oxidizing organic matter effectively, rupturing both C-P and C-O-P bonds, and in solubilizing suspended material to release the phosphorus as soluble orthophosphate. Three digestion methods are available. The perchloric acid technique is time-consuming method and is suggested for only particularly difficult samples, such as sediments. The nitric acid-sulfuric acid technique is recommended for most samples. However, the persulfate oxidation technique appears to be the simplest digestion method.

Three methods are described to determine orthophosphate concentration. Selection of a method depends largely upon the concentration range of orthophosphate. The vanadomolybdic acid method is most useful for orthophosphate analyses in the range of 1 to 20 mg P/l. The stannous chloride and ascorbic acid methods are more suited for the range of 0.01 to 6 mg P/l.

Detailed procedures for the above tests are described in Standard Methods for the Examination of Water and Wastewater, 1975 (Standard Methods for the Examination of Water and Wastewater, 1975).

5.5.11 Oxygen Management of Groundwater

Oxygen management of the groundwater system is dependent upon existing levels of DO, BOD, and COD (Buckingham, 1981). If dissolved oxygen is low and chemical and biochemical oxygen demands are high, the water requires the addition of oxygen.

It is difficult to quantify the oxygen requirements of the groundwater system. One method is described, which was specifically evaluated for an oil spill (Jamison, Raymond, and Hudson, 1975). It is based upon the composition of bacterial cells in terms of oxygen and on the conversion rate of hydrocarbons to cells. See Appendix A, Section 5.1.2.4.

There are a number of existing devices that can be used to meet the oxygen requirements of bacteria in contaminated groundwater. Fluid and semisolid systems can be aerated with pumps, propellers, stirrers, spargers, sprayers, and cascades. A system has been described, which introduces air into wells by a diffuser tube connected to a small air compressor by a rubber hose. The compressor theoretically delivers around 0.07 m^3 of air per min. Diffusers are positioned 5 ft from the well bottom. Compressors are fitted with pressure gauges and relief valves to aid in determining that each diffuser is operating properly.

5.5.12 Nutrient Management of Groundwater

Phosphorus and nitrogen are major nutrients essential to a groundwater fertilization program. They are added to the groundwater in compounds available as commercial fertilizers.

Expense plays a major consideration in selection of the type of fertilizer to add to a groundwater system (Brady, 1974). Production costs and application methods affect the overall economic feasibility of a groundwater fertilizer program. For example, low labor costs are associated with the application of liquid fertilizers. Materials are handled in tanks and pumped out for transfer or for application. Generally, cost per unit nutrient element is higher due to the demand for more sophisticated equipment for fertilizer storage and handling.

Fertilizers must be water soluble in order to be effective in releasing nutrients to the groundwater. Some fertilizers have a coating that times them for slow release into the environment. This feature is undesirable in the case of groundwater contamination because it is important to stimulate bacteria as quickly and efficiently as possible. Monitoring the nutrient levels, or other

indicator of nutrient status (such as BOD) is required so as to be able to adjust nutrient input accordingly.

Safety is another major consideration in the selection of fertilizers. Some, such as ammonium nitrate, must be handled with caution because of their explosive hazard. In addition, for long-term treatment, it is extremely important that fertilizers be of food-grade quality, since treated groundwater may ultimately reach a public drinking system. This will aid in preventing unnecessary contamination.

It is probable that subsurface injection is economically feasible when compared with methods of surface application (Smith, McWhorter, and Ward, 1977). Greater capital costs are assumed, but labor costs are reduced because of the limited surface area involved. Pumping liquid fertilizer through a rubber hose and into the well is a possible method of nutrient injection. This technique may cause agitation or aeration of the groundwater, which needs to be avoided only during the acquisition of water samples for oxygen analysis.

Nutrient requirements can be determined by two methods. The first is based upon the phosphorus and nitrogen contents of bacterial cells in relation to their carbon content, i.e., their C:N:P ratio (see Appendix A, Section 5.1.2.5). The second method bases the nutrient requirements on the BOD of the groundwater system; e.g., BOD:N:P = 100:5:1 (Sherrard and Schroeder, 1975). This ratio is generally applied to industrial wastewater treatment in order to remove a significant fraction of the organic matter present in industrial effluent. It requires BOD analysis of the contaminated groundwater followed by subsequent adjustments to nutrient levels.

Metabolic abilities and nutrient requirements of groundwater microorganisms vary substantially within an aquifer (Swindoll, Aelion, and Pfaender, draft). In some cases, mineralization may be limited by available inorganic nutrients. Other samples from the same aquifer mineralization may not be affected. Degradation can be more greatly enhanced by addition of multiple inorganic nutrients than by addition of single substances. Alternative carbon sources, such as glucose or amino acids, inhibit mineralization of the xenobiotic substrates, possibly because of preferential utilization of the more easily degradable carbon amendments.

Each *in situ* application is a research effort that must be customized to the particular site and contaminant characteristics (Amdurer, Fellman, and Abdelhamid, 1985). There are no generalized cases, and decisions must rely on engineering judgment. Nonuniform geological formations that impede the flow of waterborne compounds are not realistic candidates for *in situ* treatment. The greatest *in situ* success will be with a plume or a spill situation.

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